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PHENOMENA OF EMBRYOGENESIS AND THEIR SIGNIFICANCE FOR A THEORY OF DEVELOPMENT AND HEREDITY[1]

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I

IN discussing the phenomena of the process of animal embryogenesis, the observable, visible occurrences from which we may attempt to derive a theory of development and heredity, I must obviously limit myself to those changes that take place before the embryo is delineated. Since I envisage all eggs, I speak only of events common to all. Thus by definition I confine myself to events of the cleavage-period, ending with the setting off of ectoderm and entoderm as layers from each other. Study of these supports the proposition that the genesis of the embryo is to be sought in the intrinsic character and make-up of the egg. An egg is as specific as the organism which develops out of it. Whilst it may be true for some eggs that the mineral-content increases by additions from the external medium, this is not the case for all eggs. It is not true for eggs said to show such additions that all their minerals increase. On the other hand, it is agreed that the animal egg during cleavage gains no carbohydrate, lipin or protein from its inorganic (or

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organic) surroundings. The genesis of the embryo out of the uncleaved egg, itself a most highly differentiated system, thus represents a transformation of the egg's materials within the vitelline membrane and especially of its hyaline ground-substance. These visible events of cleavage are easily followed under the microscope.

As preface to the exposition of these phenomena, I call attention to one not directly observable event whose occurrence can be demonstrated by simple experiment. I refer to the fact, never sufficiently emphasized, that all unfertilized eggs, amenable to the experiment, have capacity to produce many embryos when fragmented. Some others naturally give rise to more than one embryo. These data, on experimental and natural polyembryony and on twinning in usually monoembryonic eggs, are significant for any theory of development and heredity. The already difficult problem of embryogenesis is additively complicated by this latent capacity of an egg for multiple embryo-production.

What, on the basis of experiments which show the loss of capacity of blastomeres isolated at some stage of cleavage to develop into complete though dwarf larvae, is generally spoken of as loss of pluripotency, we should divorce from the sharp, quickly occurring change whereby the fertilized egg loses capacity for polyembryogenesis. The egg is first potentially polyembryonic, then loses this potency; as a monoembryonic system it becomes successively restricted within the domain of monoembryogenesis. Both forms of pluripotency, if one so denominates them, I can show are related to the distribution and activity of the surface-cytoplasm (ectoplasm). It is well known, through studies on the development of isolated blastomeres, that monoembryogenesis in so-called pluripotent eggs, as those of echinids, is due to the integrity of the ectoplasm.

I turn now to the visible morphological events that occur during cleavage.

II

Six capital changes appear before our eyes as we follow the cleavage of an animal egg. These phenomena are: A, the splitting of the egg into blastomeres; B, the shifting of the cytoplasmic inclusions; C, the appearance of the embryonic axis in eggs that develop into radially symmetrical organisms and the appearance of the median plane in embryos of bilaterally symmetrical organisms; D, the increase in nuclear material; E, the distribution of water; and F, the increase in the ectoplasm.

Our first task is an endeavor to evaluate each of these in an attempt to learn which one, or combination of them, may serve as the underlying factor or "cause" of embryogenesis. Thus we seek to know how far we can relate the transformation of one visible system, the uncleaved egg, into another, the embryo, through study of tangible, observable occurrences.

A. All animal eggs go through stages of cytoplasmic cleavage. This may be early, as in the majority of eggs, or late, as in the superficial cleavage of insect-eggs. A syncytium is never the sole mode of subdivision in the animal egg. Were an uncleaved egg a homogeneous system the mere *act* of cleavage could not set up differentiation. Cleavage in such a system might be thought of as setting up chambers of varying capillary dimensions favorable to reactions; but these reactions, not the splitting up of the mass, would be the cause of differentiation.

The egg shows itself as such at the moment when by morphological characters it becomes different from all other cells of the organism of which it is a part. Thus, the uncleaved egg is already a differentiated system. I do not mean by this statement to imply that I hold with the preformationists' doctrine that in the uncleaved egg the embryo exists either in the rough or as dismembered parts. I do not agree with those who look upon cleavage as the means by which prelocalized embryonic regions fall into place. That before fertilization—or stimulation to parthenogenetic development—the egg has capacity to

produce more than one embryo is strong evidence of its being a highly differentiated system. This capacity is also evidence against the theory of prelocalization of embryonic regions.

As a mono-embryonic system, the egg becomes progressively restricted during cleavage. Having intrinsically the chemical make-up on which embryogenesis depends, the egg builds the embryo on the basis of its chemical constitution. Cleavage-planes act positively, not passively as the preformationists' doctrine holds. I therefore make a distinction between cleavage as a mere act of setting up boundaries within the egg and ectoplasmic changes in cleavage as means for facilitating (and restricting) chemical reactions within the individual blastomeres.

That cleavage as a passive act of partitioning is not *per se* the cause of embryogenesis is indicated by the reports on differentiation without cleavage in eggs of annelids and in the egg of an ascidian having had experimental treatment. The account of differentiation without cleavage in the egg of *Chaetopterus* (Lillie) warrants the conclusion that ectoplasmic changes play a deciding rôle in embryogenesis. The aberrant development without cleavage in this egg is certainly correlated with the altered state and behavior of the ectoplasm just as surely as the egg's abnormal nuclear and chromosomal phenomena parallel the changed ectoplasmic activity. With respect to the latter rôle of the ectoplasm, examples are cited elsewhere (Just, 1931). The elimination of chromosomes in the egg of *Sciara* as these approach the ectoplasm is another such example (Dubois).

B. The various spherules, granules and the like suspended in the cytoplasm are not "organ-forming materials." True, in many eggs they come to lie in definite locations. But eggs in which these cytoplasmic inclusions have been displaced by centrifugal force develop as normal eggs. Further, hyaline fragments of unfertilized eggs having been fertilized, as Lillie first showed, cleave

as whole eggs. The capacity of eggs to develop into embryos is thus to be sought in the ground-substance. The shifting of these cytoplasmic inclusions is initiated by changes in the ectoplasm. This being so, the shift can not be a primary cause of embryogenesis.

C. Proponents[2] of the proposition that a constant relationship exists between the polar axis of the animal egg and the median plane of the embryo developing therefrom have created a problem that need not exist. The problem disappears, in my judgment, the moment when we recall certain simple truths of geometry. We need first to distinguish between the polar axis in eggs of radially symmetrical animals and that in eggs of the bilaterally symmetrical with respect to the *axis* of the embryo in the former eggs and to the *median plane* in the embryo of the latter.[3]

(1) Polar and embryonic axes coincide in eggs whence radially symmetrical embryos arise. A line drawn through the egg from the site of polar body extrusion to the pole opposite is also the gastrular and the embryonic axis. In the eggs of these organisms—Porifera and Coelenterata—the gastrula forms in such wise that its axis is along *traces* of the polar axis, no matter by which of the several modes of gastrulation that obtain in these eggs the gastrula arises.[4] The gastrula may develop from a morula or a blastula; the gastrulation may be that of invagination, delamination or epiboly; finally the gastrula is a two-layered radially symmetrical structure enclosing a cavity whose polar axis, now the embryonic, for the most part traverses empty space and at whose poles only are cells located which are part of the two-layered covering. The components of the axial gradient become effective along the egg's surface.[5]

[2] *Cf.* Wilson.

[3] For example, as does Conklin, 1927.

[4] For animal eggs Korscheldt and Heider make a classification comprising six types of gastrulation. Morgan, p. 211, however, makes one of these types, invagination, synonymous with gastrulation.

[5] *Cf.* Child, p. 30.

(2) Eggs of most bilaterally symmetrical animals begin their development as radially symmetrical structures and, therefore, show a polar axis. But at the moment after fertilization when bilaterality appears in such an egg, we can no longer speak of an axis. In a bilaterally symmetrical organism—egg or adult—there exists no line common to planes as in a radially symmetrical one. Here, accurately speaking, we can use only the term, *plane of symmetry*.[6]

Certain eggs out of which develop bilaterally symmetrical animals—as, for example, those of *Loligo, Hydrophilus, Amia*—reveal bilateral organization before fertilization. In these, obviously, we can not speak of a polar axis. In them, therefore, we can not relate polar axis to the median plane of the embryo even if we know that at some time in their history the eggs were radially symmetrical. The moment that bilaterality appears in these eggs there can be no axis with respect to which the parts are symmetrically arranged.

A survey of embryogenesis in eggs whence develop bilaterally symmetrical embryos does not reveal that "the axis of the egg shows a definite relation to that of the gastrula of the later embryo, and of the adult body"; or that "this relation, broadly considered, appears to be constant throughout the Bilateralia" (Wilson, pp. 1018–1019). In many radially symmetrical eggs that subsequently show bilaterality it is true that a line drawn from the site where the polar bodies lie to the opposite pole is perpendicular to one drawn along the length of the embryo. In others—*e.g.*, egg of *Amphioxus*—the intersection of these lines forms an acute angle. In eggs that are bilaterally symmetrical before fertilization, the median plane of the embryo lies in the egg's plane of bilateral symmetry.

(3) A survey of embryogenesis in the entire animal kingdom permits the conclusion that the embryo, with the

[6] But *cf.* Conklin, p. 17, who speaks of a *bent axis* in a bilaterally symmetrical gastrula.

possible exception of the mammalian, arises from the egg-surface. In the present state of our knowledge, the mammalian egg does not seem to fall in with our generalization, since its surface-cells form trophoblast. In all other eggs it follows that either embryonic axis or median plane is normal not to the core of the egg but to its surface where the embryo lies. Axis or plane appears, due to new configurations in the protoplasmic system called forth by changes in the ectoplasm. Forces come into play that bring about new alignments first in the uncleaved egg, later successively during cleavage in the individual blastomeres. These forces that alter distribution of the cytoplasmic ground-substance are intrinsic to the egg, however much the environment acting on the ectoplasm comes into play. The living blastomeres of the cleaving egg build axis, or median plane, and embryo.

D. Elsewhere (Just, 1936) I have dealt with the phenomenon of the increase of nuclear material, the synthesis of nucleo-protein, during cleavage out of the cytoplasmic ground-substance. Here, therefore, it suffices to say that in an egg, as that of any echinid, an annelid, a mollusc or of *Amphioxus* which hatches beneath our microscope, we can follow from first cleavage onward the progressive increase in total nuclear volume and mass as the extra-nuclear cell substance decreases.

E. From fertilization through cleavage eggs of marine animals certainly, and all others probably, show a visible altered distribution of water. This is best observed in experimentally treated eggs, but it can also be seen in normal development. After the initial ectoplasmic dehydration which occurs when eggs are fertilized or subjected to experimental means that initiate development, the egg establishes a new equilibrium with the sea water. On this level water, *as discrete drops,* move from place to place within the egg or from egg to external medium. The formation of water-drops is a rhythmical phenomenon which accompanies each division-cycle of the cleavage-period.

F. During cleavage as the protoplasmic mass is sundered by cleavage-planes, the surface-area of the egg is increased. This surface-cytoplasm also displays activity which differs from one cleavage-cycle to another as with each phase of a cycle. We owe much that we know concerning the activity of the egg-surface to Mrs. Andrews' work.

Whilst Haeckel[7] invented the term, exoplasm, now commonly, ectoplasm, the fact that animal cells show differentiation into an inner core and an outer layer of cytoplasm was known to the older histologists[8]—who gave no name to the cytoplasm at the cell-surface—as shown by their descriptions of cells of metazoa. The best account of the present century of the structure and the behavior of the ectoplasm in tissue-cells and of the rôle of ectoplasm in vital activity is that in Harrison's epoch-making work on the growth of embryonic nerve-cells *in vitro*. It has also long been established[9] that in protozoa endo-ectoplasmic differentiation is marked. Ectoplasm and its derivatives are used as a basis for classifying protozoa. Thus eggs are not the only cells whose surface-cytoplasm exhibits morphological, physical and physiological characteristics that set it off from the endoplasm. On any egg whose ectoplasm is not easily discernible I find that experimental treatment, completely reversible in its effect, can bring it out very sharply.[10] The differences in ectoplasmic structure of eggs are so sharp that this structure has taxonomic value—by it one can identify species of eggs. We may speak of a "conventional design" of ectoplasm, comprising a rim of cytoplasm whence filaments arise whose ends are covered by a delicate membrane; yet, the diameter and length of the filaments, the distance of any filament from its neighbors and the distance between the covering of the filaments and vitelline membrane, vary sufficiently among eggs

[7] But *cf.* Fol's claim.

[8] See Brücke and earlier writers.

[9] Haeckel and others.

[10] See also Fauré-Freuiet.

of different species to set any species-egg off from all others.[11]

III

Of the six capital events enumerated above I endeavor now to establish the thesis that three are responsible for embryogenesis. I dismiss loss of pluripotency not only because it is an event which we can not directly observe, although we can ascertain that it occurs, but also because, in my judgment, to speak of loss of pluripotency is merely otherwise to say that embryogenesis is a series of progressively restricted changes; speaking thus, we but restate our problem, although now on the basis of experiment we express ourselves more dramatically.

Any unfertilized egg whose fragments can each produce an embryo is, whilst intact, theoretically polyspermic. From the moment when such a normal egg reacts with a single spermatozoön, it becomes monospermic and loses the pluripotency of its unfertilized state. The subsequent history of the egg—whether it develops as either normally monoembryonic or polyembryonic or, being normally monoembryonic, it, due to experimental treatment, gives rise to several embryos—reveals further loss of pluripotency. This loss is different from that sustained by the egg when it reacted with a single spermatozoön. And yet the loss of pluripotency at fertilization indicates to us at once to what we may relate the restriction which progresses with cleavage. The explosion at the egg-surface following sperm-contact with egg because of which part of the ectoplasm disintegrates with momentary loss of equilibrium between egg and medium is a crashing prologue to the acts of the drama to follow

[11] Ectoplasm has been described for many eggs by many workers. A few are named: E. A. Andrews, G. F. Andrews, Appellof, Bergmann, Berthold, Bohm, Bütschli, Calberla, Chun, Fol, Gatenby, Gerould, Goldschmidt, and Popoff, Groom, Herbst, Haeckel, Hammar, C. W. Hargitt, His, Janicki, Korotneff, Kostanecki, Kowalewsky, Lang, Lewis and Hartmann, List, Maas, Metchnikoff, Meves, Pereyaslawzewa, Provho, Selenka, Sobotta, Spengel, Storch, Theel, Vejdowsky, Warren, C. B. Wilson, Yatsu, Zelinka, Ziegler. Every phylum of Metazoa is represented in these descriptions.

and gives more than a hint to the plot of the piece. Since Fleming's and Peremescho's writings we have known that with cell-division ectoplasmic changes occur. These are but minor variations of those which follow contact of spermatozoön with egg.

Three of the six phenomena enumerated I do not regard as contributing causes to the genesis of the embryo, although in each the ectoplasm plays a rôle. There remain: the increase of nuclei, the distribution of water, and the increase in ectoplasm.

The ground-substance elaborates nuclei. Egg-fragments, composed of this same ground-substance only, if fertilized, develop into embryos. Nuclear synthesis is a repetitive process parelleling the cleavage-cycles. It tends to exhaust the ground-substance as the restriction during embryogenesis progresses. The synthesis is that of a definite stuff, nucleo-protein, specific for the organism; its result is ever visible for nuclei are morphological structures. As such the result of the synthesis differs from that of many another taking place in cells. This striking elaboration of nuclei during cleavage permits us to conclude that a cause of embryogenesis is to be sought in the chemical changes underlying and attending the synthesis.

What is left in the cytoplasmic ground-substance at the close of each cleavage-cycle is not only the source of nuclear material for the next cleavage but also the domain where occur changes by which we recognize the differences between a blastomere of one cleavage-stage and that of another stage.

These changes are in part, certainly, chemical—some, like the elaboration of nucleo-protein, are syntheses; some are hydrolyses. We do not directly observe these chemical reactions which take place in the ground-substance. But we can directly observe that water shifts from place to place during a cleavage-cycle and that its distribution varies from one cleavage-stage to the next. We know that water plays a rôle in all reactions of cells. The dis-

tribution of water in the blastomeres is thus a factor which determines the chemical reactions that lie at the basis of embryogenesis, as it goes far toward determining the physical attributes of living substance.

The addition or removal of water in a reversible reaction determines hydrolysis or synthesis, respectively. Thus, water plays a rôle as a component in chemical reactions; in addition it is both a solvent for other components and a part of the cytoplasmic structure, the chamber of the reactions. Then the demonstration that water is intermittently present as discrete drops in viable cells having had any one of several kinds of treatment—whose action is not deleterious since one can prove that the cell after treatment returns completely to the untreated state—has tremendous significance: it becomes an index to the nature and rate of reactions. In both normal egg and blastomeres, the rhythmical appearance of water-drops allows us to correlate their occurrence with the rhythm in a cleavage. Differences in the formation of the water-drops in different blastomeres we may regard as an index to chemical changes underlying the progressive restriction that runs with cleavage.

With each succeeding cleavage the egg is further subdivided into chambers of capillary dimensions. At the end of the cleavage-period the total surface-area of the blastomeres is much greater than that of the uncleaved egg. The chambers vary in size, shape and relation to each other. These attributes alone could account for differences between the reactions within one blastomere and another. What accounts for these variations in size, shape and relation is the ectoplasmic activity. The cleavage-walls are not mere inert structures; they show intense activity, a spinning of filaments, as Mrs. Gardiner so clearly described, an activity as intense and as significant for the blastomeres as that described by Harrison for the growth and differentiation of embryonic nerve-cells. The differential activity of the ectoplasm thus conditions the reactions within the cleaving egg.

In more direct manner it can be shown that the ectoplasm sets the stage for intracellular activity. Here I refer to visible morphological changes. Note Lillie's account of the relation between altered ectoplasmic structure and chromosomal behavior in cleavage without differentiation; Dubois' finding (p. 604 and p. 612) concerning ectoplasm and chromatin-elimination in the egg of *Sciara;* my own observation on ectoplasmic injury as the cause of polyploidy in the egg of *Nereis* (Just, 1933).

IV

The progressive increase of nuclear and of ectoplasmic material are two changes that occur during cleavage to which as causes I relate embryogenesis. With these I place the alternating hydration and dehydration of the protoplasmic system, a rhythmical phenomenon in the cleaving egg whose surface at fertilization underwent rapid hydration and dehydration. From then onward the egg establishes new equilibrium with its medium with each cleavage-stage.

At the beginning and at the end of cleavage when ectoderm and entoderm are laid down the egg is a morphological entity. In the endeavor to answer the question—how out of one visible structure, egg, arises another visible structure, embryo?—I have dealt with visible structural changes which take place between beginning and end-stages. These form-changes arise from activity in the cytoplasmic ground-substance. Hence, I relate the origin of the tangible embryo to tangible cytoplasmic changes. The cytoplasm builds the embryo. Then it builds all of it, including characters called Mendelian.

Certain protoplasmic systems, syncytia, contain many nuclei within the protoplasmic boundary. Others, *e.g.,* among bacteria and blue-green algae, are without discrete nuclei, although they doubtless contain nuclear stuff.[12] Once it is demonstrated that ultra-filterable viruses are

[12] See A. J. Schaffer, C. Folkoff, and S. Bayne-Jones, The Johns Hopkins Hosp. *Bull.,* 33, 1922.

living things, we may begin to speculate concerning their organization as protoplasmic systems. But to me as a morphologist, these considerations although appreciated do not apply to my object of study. For the egg is that protoplasmic system, a cell, which comprises nucleus and cytoplasm. Until I know of one egg, or fragment thereof, which, lacking this cellular organization, can develop into a demonstrably living larva, it is idle for me to speak of any other unit than that capable of development. No component of the egg, cytoplasm or nucleus—and certainly no isolated gene standing alone, ever develops into an embryo. Nucleo-cytoplasmic organization for the morphologist dealing with the cell as here defined is the biological unit. (But *cf.* Jennings.)

I do not here advocate a return to old-fashioned morphology. We can not return to that from which we have not yet escaped. Not until we have exhausted the study of every cell-component by descriptions as thorough as those on record for the chromosome can we disregard investigations on the form and form-changes in the cytoplasm. We need to-day a concentrated attack on the ground-substance of eggs, whence arise nuclei, which builds up endo-ectoplasmic differentiation and where water, quantitatively the most important compound in cells, forms in drops. Out of the ground-substance the embryo arises—every part of it to its least inconsequential character. This is my theory for development and heredity.

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THE GENETIC CONTROL OF DEVELOPMENTAL RELATIONSHIPS[1]

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IN the very difficult problem of determining the mechanism by which genes control development and determine those traits in the adult by which genic differences are recognized, the first step toward a solution must evidently be to describe exactly the course which such traits follow in development. Just *what* a gene controls must be learned before we can understand *how* the gene controls it. Unfortunately in many of the traits best known genetically, notably differences in color, such a procedure is almost impossible at present because of the difficulty of tracing more than the last few steps in the chain of reactions between gene and character. Traits of form, however, offer peculiar advantages in such a problem, since many of them may be observed and measured throughout practically the entire course of their development. Especially favorable material is provided by the multiple organs of plants, such as leaves and fruits, for large numbers, genetically identical, may be obtained from the same individual.

In the family Cucurbitaceae (the squashes, gourds, melons and similar plants) there is a very great diversity in fruit shape, and in several of its members, notably *Lagenaria vulgaris* and *Cucurbita Pepo,* this occurs even within a single species. In a wide range of types, measurements have been made of the length (polar diameter) and width (equatorial diameter) of ovary and fruit from very early primordia, microscopic in size, to maturity.

[1] Paper presented in a discussion session on Genetics and Development before the Genetics Society of America in a joint session with the American Society of Zoologists and the American Society of Naturalists at Atlantic City, December 30, 1936.

Marked differences were observed in the way in which the shape of the mature fruit developed.

In *Lagenaria,* the earliest ovary primordia are all much alike and the marked differences in fruit shape which later arise are due to differential growth of the dimensions throughout the whole course of development. In the bottle gourd, for example, growth in width is slightly but steadily more rapid than growth in length, so that the fruit becomes progressively flatter as it grows larger. In the Hercules club, on the other hand, length growth is constantly more rapid than width growth, so that fruit shape becomes more and more elongate until the attenuated club-like mature fruit is developed. The remarkable fact in both these cases is that this growth relationship is of the type now commonly known as *heterogonic* (*allometric*), since the growth rates of the two dimensions (in the sense of percentage or "compound interest" rates) maintain the same relationship to each other throughout. This is shown graphically by the fact that when the logarithm of length is plotted against that of width, the points fall along a straight line. Although the actual ratio of length to width may thus change markedly with increasing size, the fundamental growth relationship is constant. If in the formula for heterogony, $y = bx^k$, we let y represent length, and x width, then the value of k, represented graphically by the slope of the plotted line, serves as a measure of this growth relationship. In the bottle gourd, k is .8, indicating that length is growing only about .8 as fast as width. In the club, on the other hand, k is 1.2, indicating the amount by which growth rate in length exceeds that in width. The value of k in the family as a whole ranges from about .8 to an extreme of 2.2 in the snake gourd, *Trichosanthes,* which at maturity is about 50 times as long as wide. In some races there is a sudden change in the slope of the line at a definite point late in development, but the line is straight on both sides of this point.

The significant fact for our purposes in all this is that the trait of shape (so far as it is measured by a single ratio) may be expressed for each of these races by the value of the relative-growth constant k. To be sure, even in races where length and width fall along the same line in development, there may still be marked differences in fruit shape at maturity depending upon the size which the fruit ultimately attains; but the fundamental shape difference is the *slope* of the line, not its length. That a character may thus be described by a single constant, unchanging throughout development or for very long periods and independent of size, is evidently an important step toward a simpler statement of what is actually inherited. The conclusion that in cucurbit fruit shape this value is genetically controlled is further indicated by the fact that the size of the relative growth constant for length to width clearly segregates after crosses between types in which it differs.

In other members of the family, such as *Cucurbita Pepo,* there is much less variability in the relative growth constant, which has a value of between .9 and 1.0. In other words, shape changes little during development. The marked differences which occur here are in the *level* of the length-width line rather than in its slope, and thus are measured by the value of the constant b in the heterogony formula. These differences are established early in development, although not at the very beginning. If the earliest primordia are examined they are all found to be essentially alike; but shortly after growth begins, some races show a marked increase of length over width, others of width over length, and others are unchanged. This differential growth can not be very accurately measured but seems also to be heterogonic. It persists for only a short time, though long enough to establish the primordia at different indices or levels, after which the dimensions grow at nearly the same (percentage) rate. In this species the period during which shape differences arise does

not extend over all (or nearly all) the developmental history, as in *Lagenaria,* but seems to have been pushed back into the very early stages. In both cases, however, there is the same constancy of relative growth rate for long periods or throughout development. Detailed evidence has elsewhere been presented[2] that it is dimensional *relationships* which are inherited here rather than absolute dimensions themselves.

Of course fruit shape is not a single ratio but an entire pattern or integrated series of length-width ratios at various levels along the axis. Patterns are certainly inherited. For example, there are two races of *Cucurbita Pepo* in which the fruits are essentially isodiametric but with very different profiles or patterns, each of which differs from the "disk" fruit shape by a single genetic factor. The extracted isodiametric segregates from crosses of these lines with disk types resemble in pattern the particular type used in the cross. Pattern differences arise very early. Whether the complex relationships here involved originate from the differential growth rate of dimensions or through some specific localization of growth potencies can not readily be determined on account of the small size of the structures involved.

It remains true that in all cases where the origin of shape differences can be followed, each is the result of a constant and specific relative growth rate between its major dimensions. This same constancy may be seen in other aspects of fruit development. Thus when width of pericarp is plotted against width of placental region, the former is found to grow about .75 as fast as the latter, so that as the fruit increases in size, its pericarp becomes relatively thinner. Length of seed, however, grows much faster than width of fruit, the value of the constant here being about 1.3 in most races. Dimensional growth in the fruit stalk shows even more extreme differences, length growing very much faster than diameter. In all these

[2] E. W. Sinnott, *Genetics,* 20: 12–21, 1935.

cases, the same straight-line relationship appears, with various values for slope and level (values of constants k and b in the heterogony equation). The same relationship is found commonly, if not invariably, between dimensions of developing leaves and has been reported for a number of other plant traits; and for animal material the literature of heterogony is now extensive. It seems probable that wherever complicating conditions are absent this relationship holds for all structures and dimensions of a developing organism. However this may be, the fact that such constancies in relative dimensional growth do occur makes it possible to express the differences between morphological characters much more simply and exactly than by a description of their mature aspects alone. What is inherited, and therefore what genes control, seem to be these constant growth relationships. As growth proceeds, the proportions of parts change, complexity increases, and the familiar developmental story unfolds. Running through all this complexity, however, is a basic constancy, the inherited growth relationship, established from the beginning. It should be possible to determine for any organic pattern a series of constants of this sort from which one could predict its form at any size or stage. These constants would be the real expression of genic activity, or the true *character* itself.

Such a developmental analysis, important as it is in stating very precisely *what* the genes control, gives no information as to *how* the control is exercised. Its results, however, suggest a few tempting speculations.

If what a gene controls is a growth relationship and if this is constant during development, it seems very likely that the gene itself may be operating constantly and in the same manner. The fact that a series of profound changes in form occurs during development thus would not require a chain of successive and different causal processes but would merely be the necessary result of an increase in size where the genotype determines constant differences

in relative growth. Genes may not only initiate such a developmental history but may exercise a constant control, unchanging in character, until the process is completed.

If genes control relationships between rates of growth in various dimensions, they may perhaps control relationships between rates of other developmental processes which are not spatially arranged, notably the complex series of chemical changes concerned in the development of many traits. This is opposed to the view that the gene initiates only the first step in such a series, the later ones bearing no resemblance to the original genic impetus. The remarkable instances of what Needham and others have described as "chemical heterogony," where during development there is a constant relationship between the rate of increase of a particular substance and that of the rest of the organism, suggest that there may be a constantly operating mechanism for controlling relationships between rates of chemical change here and that direct genic effects are thus not limited to the first steps in the series. There may be, in other words, a true "pattern" of chemical reactions which in its underlying control may be somewhat like a morphological pattern.

The relationships here discussed are quite independent of time or any absolute rate of change, and they express themselves equally well in rapidly growing and in slowly growing organisms. The view that genes control these relationships is therefore somewhat different from the one now commonly held, which assumes that they determine the absolute rates of particular processes. Every one will probably agree that, whatever the mechanism of gene action is, an individual can not well be a congeries of independent and unrelated gene-controlled activities. Some agency knits these together and integrates them into an *organism*. The physical basis underlying this organization is unknown, but the fact of organization itself can not be ignored in studying biological processes. In any organized entity, therefore, it is difficult to see how the rate

of one process can be altered without affecting the rest. It may reasonably be assumed that every genic change, whatever its immediate and specific effect, alters the whole organized pattern to some extent, and that this alteration can be more completely described in terms of altered relationships than merely of an altered rate. Genes may thus be looked upon as regulators or units of organization rather than as activators of specific processes. It is altogether probable, of course, that they have various types of effects.

Since the mechanism of gene action is still entirely unknown, such speculations are not particularly fruitful. What is first needed before we shall be ready for the ultimate attack on the nature of the developmental mechanism in physiological terms is a much larger body of descriptive information about the exact course of development, both as to rates and relationships between them. The extent of our knowledge in this field is still extremely meager. At present such information can probably be obtained more readily than elsewhere from a quantitative study of the development of morphological characters.

THE DEVELOPMENT OF EYE COLORS IN DROSOPHILA AS STUDIED BY TRANSPLANTATION[1]

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INTRODUCTION

THE present report is an attempt to summarize briefly studies of eye color development in *Drosophila melanogaster*, carried out, for the most part, jointly by Dr. Boris Ephrussi of the Institut de Biologie physico-chemique, Paris, and the writer. Detailed evidence for statements made here have been or will be published elsewhere (see references at the end of this paper).

Those developmental reactions leading to the formation of specific types of eye colors in *Drosophila* presumably constitute a very small part of the general reticulum of developmental reactions. This particular small group, which of course in reality is probably very complicated, has several advantages for experimental study: (1) the genetic basis of eye color inheritance is relatively well understood; (2) eye colors have convenient characteristics which presumably can eventually be expressed in terms of definite chemical pigments (see Schultz, 1935); and (3) experience has shown that the reactions are approachable with a simple technique of transplantation (see Ephrussi and Beadle, 1936a, and Beadle and Ephrussi, 1936).

DIFFUSIBLE SUBSTANCES AND EYE COLOR

A series of eye-transplant experiments has indicated the existence of three specific diffusible substances, all of which are necessary for the production of wild-type eye color. The names assigned to these and their characteristics are as follows:

[1] Paper presented in a discussion session on Genetics and Development before the Genetics Society of America in a joint session with the American Society of Zoologists and the American Society of Naturalists at Atlantic City, December 30, 1936.

(1) *ca+ substance*—a substance which a claret (a pink eye color) host can not supply to a genetically wild-type implant.
(2) *v+ substance*—a substance which will result in the production of wild-type eye color when supplied to a genetically vermilion (a bright red eye color) eye.
(3) *cn+ substance*—a substance which when supplied to a genetically cinnabar (a bright red eye color, like vermilion) eye results in the production of wild-type eye color.

From experiments described by Ephrussi and Beadle (1937c) and by Beadle and Ephrussi (1937a) it has been concluded that these three substances are interdependent in some manner such that the relationship can be represented by a succession of three steps in a linear series, thus

$$\rightarrow ca^+ \text{ substance} \rightarrow v^+ \text{ substance} \rightarrow cn^+ \text{ substance.}$$

It is assumed that ca^+ substance is somehow essential for the formation of v^+ substance and that, similarly, v^+ substance is necessary for the formation of cn^+ substance. There is no sound basis for assuming that this interdependence in formation is referable to a direct chemical transformation of one substance into the one next in the series.

The evidence for the existence of three distinct diffusible substances and also the evidence for the sequential relations as indicated will be found summarized in the papers referred to above.

Nature of the Diffusible Substances

Howland, Glancy and Sonnenblick (1937) have found that wild-type flies of *Drosophila melanogaster, D. simulans, D. pseudoobscura* and *D. virilis* all produce v^+ substance which is active in changing vermilion eyes of the same species and also in changing vermilion eyes of any of the other species in this group. Ephrussi and Harnly (1936) have shown that Galleria and Calliphora pupae contain substances active in modifying vermilion and cinnabar eyes of *D. melanogaster;* presumably these substances are identical with the v^+ and cn^+ substances of

Drosophila. These results suggest that these two substances are non-specific in nature and that they are widely distributed in insects.

Working with pupae of Calliphora, Khouvine, Ephrussi and Harnly (in press) have shown that v^+ and cn^+ substances are insoluble in ether but are soluble in 96 per cent. ethyl alcohol-ethyl ether mixtures and in 96 per cent. ethyl alcohol. Thimann and Beadle (in press) have obtained essentially similar results. It is found that these same two substances extracted from *Drosophila* pupae are insoluble in acetone and in sesame oil but are readily soluble in water. Both substances are heat-stable at 100° C., can be freed of proteins and are apparently inactivated by enzymic oxidation. It seems clear from the above facts that the two substances are not enzymes and are not proteins.

Time of Appearance of Substances in Body Fluid

Transplants of wild-type eye anlagen to claret hosts at different stages of development have shown that the wild-type eye must normally take up ca^+ substance at a stage of development reached during the period of 24 hours prior to puparium formation (Ephrussi and Beadle, 1937d). On the other hand, v^+ substance appears not to be present in the body fluid in appreciable quantities until after puparium formation (Ephrussi, Clancy and Beadle, 1937); it is apparently present in the body fluid throughout most of development after puparium formation. The workers just cited have shown that vermilion eyes are sensitive to the addition of v^+ substance during a period extending from the late larval stage (or possibly earlier) to a stage reached at about 80 hours after puparium formation (at 25° C.).

Source of Diffusible Substances

It is evident from the manner in which it is detected that ca^+ substance is not produced in the eye. Tests for its

production by wild-type ovaries have given negative results (Ephrussi and Beadle, 1937d). Aside from this negative information, nothing is known as to the source of ca^+ substance in the fly.

Sturtevant's experiments on vermilion-wild-type mosaics in *D. simulans* (1932) can be interpreted as indicating that v^+ substance must be produced by wild-type eye tissue itself. Wild type implants grown in vermilion hosts develop wild type eye color; this is interpreted in a similar manner—formation of v^+ substance by the wild-type implant (Beadle and Ephrussi, 1936a). Eyes of certain genetic types have been shown to release v^+ substance (Ephrussi and Beadle, 1937b). Ovaries give negative results in tests for v^+ substance (Ephrussi and Beadle, 1935), as do salivary glands (Ephrussi, unpublished, and Beadle, in press). Both fat bodies and Malpighian tubes of wild-type flies have been found to liberate v^+ substance (Beadle, in press). Apparently any part of the fat body is active. It makes no difference whether an ovary is transplanted simultaneously with the fat body or not. Fat bodies are active when transplanted about 40 hours before puparium formation or when transplanted shortly before puparium formation.

By transplantation experiments it has been shown that cn^+ substance, like v^+ substance, is produced by wild-type eye tissue (same references). Ovaries give negative results in tests for this substance (Ephrussi and Beadle, 1935 and unpublished). More recently salivary glands, gastric caeca and that portion of the hind gut regenerated by its imaginal ring (see Robertson, 1937) have also given negative results (Beadle, in press). In contrast to the tests for v^+ substances, wild-type fat bodies give no test for cn^+ substance. On the other hand, wild-type Malpighian tubes do liberate cn^+ substance, even though they are transplanted as early as about 24 hours before puparium formation. Thus far, then, only eye tissue and Malpighian tubes are known to produce cn^+ substance.

RELATION OF VARIOUS GENES TO DIFFUSIBLE SUBSTANCES

From transplantation experiments of various kinds it has been assumed that many different genes must be concerned with the production of the postulated diffusible substances. For example, in a cinnabar fly, which differs genetically from wild type at a single gene locus, cn^+ substance is deficient in amount as compared with wild type. Since, aside from the color of its eyes, a cinnabar fly is essentially normal, it is assumed that the normal allele of the cinnabar gene is in some way more or less directly necessary for the production of this diffusible substance. As to how direct this relation may be and as to precisely what the relation is, no information is available. For the sake of a simple working hypothesis it may be assumed that one or more products of the normal allele are not produced by the particular mutant allele known as cinnabar and that these are necessary for the reactions leading to the production of cn^+ substance.

Numbering the steps in the sequence of substances

$$\underset{(1)}{\rightarrow ca^+ \text{ substance}} \rightarrow \underset{(2)}{v^+ \text{ substance}} \rightarrow \underset{(3)}{cn^+ \text{ substance}}$$

and making assumptions similar to those just considered for cinnabar, it has been postulated that the normal allele of the claret gene is concerned with step 1, that of vermilion with step 2 and that of cinnabar with step 3 (Beadle and Ephrussi, 1937a). No specific assumption need be made as to the number of reactions actually represented by each step in this scheme.

Considering other eye color genes, several types of experiments have indicated that many of them are concerned in the production of these substances. By testing directly for the presence of v^+ and cn^+ substance by growing vermilion and cinnabar implants in various eye color mutant hosts (Beadle and Ephrussi, 1936a), it has been found that carnation, carmine, garnet-2, peach and ruby flies are characterized by reduced amounts of v^+ and cn^+ substances as compared with wild-type flies. The normal

alleles of the genes associated with these eye colors, then, are assumed to be concerned with step 2 in the sequence. In a similar way, the eye color mutants bright and mahogany are found to be deficient in only cn^+ substance, indicating that the normal alleles of the bright and mahogany genes have to do with step 3.

By growing eyes of the various mutant types in vermilion hosts Ephrussi and Beadle (1937a) were led to conclude that the amounts of v^+ or cn^+ (or both) substances formed by bordeaux, clot, Henna-recessive, purploid (?), prune-2, purple, raspberry-2, sepia, sepiaoid and safranin-2 eyes are less than the amounts they use in their normal positions. It may be assumed that the normal alleles of the genes differentiating these characters are concerned with either step 2 or 3. In a similar way there is evidence that the Bar gene (known to be a duplication and normally not influencing eye color) influences the production of v^+ substance in the eye, indicating a relation to step 2.

Under specific genetic conditions, implanted eyes release diffusible substances which can be detected by effects on the eyes of the host. In such release, a scarlet eye (also others) has been shown by Ephrussi and Beadle (1937b) to differ from a wild-type eye.

The recessive gene suppressor of vermilion brings about a change such that measurable amounts of v^+ and cn^+ substances are produced by a fly homozygous for the vermilion gene (Beadle and Ephrussi, 1936b); presumably this gene has something to do with step 2.

Beadle (in press) has shown that Malpighian tubes from the eye color mutants light, maroon and white produce less cn^+ substance than do wild-type Malpighian tubes.

From the above it can be seen that 24 eye color genes are somehow concerned with the production of one or more of the three diffusible substances. Of the 26 eye color mutants which have been worked with, only two, brown and cardinal, have not been shown to differ measur-

ably from wild type in their diffusible substances; in these two, as a matter of fact, there are indirect indications that the diffusible substance relations differ from wild type. Thus it is seen that it is a fair assumption that any mutation that brings about a visible change in the eye color of the fly will alter in some way the production of one or more of the three eye color diffusible substances for which we have evidence.

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THE INFLUENCE OF NUCLEAR FACTORS IN HYBRID DEVELOPMENT STUDIED BY TRANSPLANTATION[1]

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THE differences in objectives which have sometimes seemed to distinguish embryological and genetic experimentation are often more apparent than real. Although the genetic motive is usually not explicit, the many studies on embryonic organization are essentially attempts to interpret the mode by which hereditary features of the organism gain expression during development. The genetic import is of course more direct when tissue is transplanted between different species, but the specificity factors thereby revealed constitute only one aspect of the complex developmental process. Their rôle is largely climactic, in shaping the specific features of a pattern whose first inception depends on the equally fundamental components of activation and organization. Although this distinction is to some extent arbitrary, it is nevertheless true that any visualization of the mode of hereditary expression is incomplete if confined to the factors controlling terminal, *i.e.*, specific, features of development.

For studies of this borderline nature the amphibians combine both advantages and disadvantages. Although the primary factors in embryonic organization are incompletely understood at best, these animals have provided, and continue to offer, perhaps the most suitable material for their study. This advantage applies not only in the analysis of general features of development, but also to the study of specific form by hetero- and xenoplastic transplantation. The principal disadvantage of the amphibians, and one which can probably be overcome only in slight measure, is their unsuitability for strictly genetic

[1] Paper presented in a discussion session on Genetics and Development before the Genetics Society of America in a joint session with the American Society of Zoologists and the American Society of Naturalists at Atlantic City, December 30, 1936.

analysis. Although one does not question the genic basis of their species pattern, it is unlikely that we can ever gain an intimate picture of the arrangement of genic loci within the nucleus. This will continue to obstruct any attempt to localize the ultimate hereditary units responsible for any given specific trait whose developmental expression we may wish to investigate. One is forced to deal with the nuclear complex as a whole instead of with its known parts. Considering the present state of our knowledge, however, this limitation is less serious than it may seem. Even when the chromosomal pattern is known, the question of genic interaction is so complex that one is almost forced to a simple formulation of the problem of developmental genetics.

The general plan of the present investigation has been to alter the nuclear constitution of the amphibian egg by hybridization and then attempt to identify the ontogenetic factors responsible for the ensuing modifications of development. This program is the outgrowth of a taxonomic study of the western Newt, *Triturus,* which revealed material unusually well adapted for the purpose at hand. The first advantage is the range of genetic types available. The genus is represented in California by three distinct species (Twitty, 1935), and more recent discoveries have added two forms probably of varietal or sub-specific status. There are indications, moreover, that this number will be extended by further exploration. A second feature of importance is that the various forms are distinguished by very clear-cut differences, and the fact that this applies particularly to the earlier developmental stages is in itself of considerable value. Most crucial of all, obviously, is the fact that all the combinations attempted submit very successfully to artificial hybridization. Sufficient time has not yet elapsed to determine whether the hybrids will yield F_2 generations.

I. PIGMENTATION

Fig. 1 illustrates some of the differences in larval pigmentation between the five forms of *Triturus.* It will be

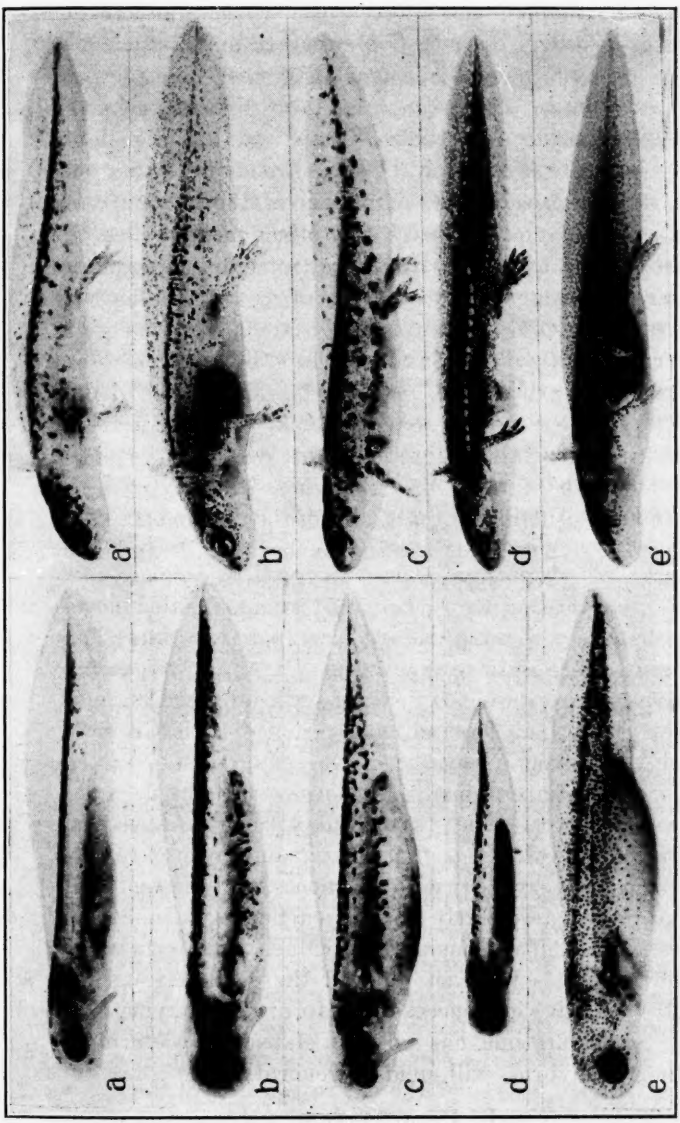

FIG. 1. Illustrating the pigmentation of the various forms of Californian *Triturus*. *a*, *a'*, *T. torosus*, young and old larvae respectively. *b*, *b'*, geographical variant of *T. torosus* from the Santa Lucia Mountains, Monterey County. *c*, *c'*, variant of *T. torosus* from the vicinity of Chico, California. *d*, *d'*, *T. similans*. *e*, *e'*, *T. rivularis*. (Size differences in the young specimens, approximately as shown in the photographs.)

noted that the principal differences consist in the varying degrees to which the melanophores are distributed over the sides. In young *T. torosus* (Fig. 1*a*) these pigment cells are almost all concentrated into distinctive bands, one on either side of the back. The few additional melanophores usually present on the sides are confined to a narrow strip along the dorsal margin of the bulging yolk mass. If we now compare the other members of the series, in the order of their arrangement in the photograph, the reader will note that the degree of melanophore dispersion becomes increasingly greater. The change in this respect is not striking in *b* and *c*, both of which are geographical variants of *T. torosus*. The dorsal bands are nevertheless not as sharply demarcated as in *a*, and there are invariably more melanophores on the sides. Moreover, these latter cells, unlike those in *torosus* proper, may extend ventrally onto the side of the yolk mass. This difference, although not striking, is very definite and characteristic. As development progresses the divergence of pattern in *a*, *b* and *c* becomes more evident (see *a'*, *b'*, *c'*). *Torosus* continues to be characterized by persistent dorsal bands and a relative paucity of melanophores on the sides. In *b'* the bands have become less prominent, particularly in the posterior region, and the melanophores on the sides are now quite numerous. In *c'* the dorsal bands may break completely in older larvae, with the melanophores aggregating in well-distributed patches.

The increased dispersion of the melanophores noted in *b* and *c* becomes much more striking in larvae of *similans* (*d*). Although the same areas of pigmentation are to be recognized in young specimens, there are fewer melanophores, less compactly arranged, in the dorsal bands, and an increase in their number on the sides. In older larvae (*d'*) the dispersion is complete, with melanophores now densely distributed over the entire sides, with the exception of small unpigmented areas in the position of the lateral line organs.

Finally, in *T. rivularis,* we find that all evidence of segregation of the melanophores has virtually disappeared. From the first establishment of pigmentation in young embryos (*e*), as well as in advanced larval stages (*e'*), the melanophores are distributed with unusual uniformity over the entire lateral surfaces.

It should be explained that this series is arbitrarily arranged, with no implication whatever as to evolutionary relationships or sequence. Nevertheless, it is interesting to note that the outstanding differences in pigment pattern in these forms can be reduced to a single factor, namely, the degree of aggregation of the pigment-bearing cells. There are other differences, to be sure, such as in total melanophore number, but purely quantitative features of this nature contribute much less in the determination of distinctive pattern than do factors of spatial arrangement.

Space does not permit a detailed redescription of pigment development in the various hybrid combinations (Twitty, 1936). It is sufficient to explain that in general the male influence results in a compromised distribution of the melanophores. For example, *torosus* sperm always decreases, but does not entirely overcome, the dispersion of the melanophores characteristic of the normal maternal species. Conversely, *rivularis* sperm brings about a partial disruption of the more circumscribed melanophore aggregations normally found in the other species. The resulting hybrid pigment patterns, since they are all of this intermediate character, unite even closer the members of the normal parental series shown in Fig. 1, into a finely graded sequence beginning with extreme localization of the melanophores and terminating in extreme dispersion of these cells.

Any attempt to analyze the manner in which the nuclear factors produce these alterations in pigment pattern must be founded upon a knowledge of the basic facts concerning pigment development. In view of the large amount of attention given to problems of pigmentation in the past, it is interesting to note that the most crucial question of

all, namely, the embryonic origin of the chromatophores, has been answered only very recently. DuShane (1934, 1935), corroborated by others, has demonstrated that in amphibia these cells originate as part of the ganglion crest. As development proceeds the prospective melanophores move from this source along the dorsal mid-line and assume the position and arrangement characteristic of the species. Another fact of cardinal importance is that the actual formation of pigment within these cells is dependent upon some stimulus or material contribution from the overlying ectoderm (Harrison, 1935; DuShane, 1935). Recent experiments with *T. torosus* have revealed the operation of still another type of factor in pigment development. Granted the presence of prospective melanophores, as well as the ectodermal factors essential for melanin elaboration, it has been shown that the movements of these cells, and hence the pattern which they form, is influenced by their topographical relationships to neural tube and mesodermal somites (Twitty, 1936).

This brief outline is sufficient to suggest the complex nature of the developmental process by which the definitive larval pigmentation is attained. The bearing of this fact on the present investigation is obvious. Knowing the multiplicity of factors involved, our first problem is to identify the component or components principally affected by the sperm in producing the modifications of hybrid development. In this attempt we have concentrated particularly on the influence of hybridization on melanophore arrangement, *i.e.*, pigment pattern in the strict sense. As pointed out above, the most interesting differences between the parental forms are not in melanophore number, although such differences exist, but in the manner of their distribution upon the larva.

It is believed that the evidence already published demonstrates that the developmental factors responsible for the principal differences in pigment pattern are located within the pigment-forming cells themselves. When neural crest is grafted from *T. torosus* embryos, *e.g.*, to

the corresponding position on embryos of *T. similans* or *rivularis,* the transplanted melanophores reproduce their own characteristic pattern in that region of the host (Twitty, 1936, Fig. 12). Although it had been found previously that the development of this pattern is conditioned by environmental effects exercised by the neural tube and somites, the results just cited show that these requisites are provided equally well by the corresponding structures of the host species. From this it should follow that in hybridization only the melanophores themselves will be modified by the foreign sperm, leaving the environmental conditions unchanged. This was confirmed by transplanting the crest from hybrid embryos back to the maternal environment, *i.e.,* upon a normal embryo of the species used as female for the cross. The grafted cells again reproduce the characteristic donor, namely, the hybrid, pattern.

(1) The first object of the present account is to make known the results of further experiments involving the use of hybrid embryos for transplantation. This can be accomplished briefly, since in all cases the outcome confirms the conclusions already indicated. Neural crest was transplanted in a number of combinations between normal and hybrid embryos, and invariably the grafted pigment-forming cells preserved essentially the same pattern of distribution originally intended for the donor environments. Some of these experiments are illustrated in Fig. 2, where one will find a fuller explanation appended.

In the first publication of this series an attempt was made to interpret the nature of the intrinsic factors which regulate the movements of the prospective melanophores, and which thereby account for the specific differences in pigment pattern. In all the species involved these cells begin their existence in the dorsal mid-line, as part of the neural crest, but in subsequent development there are striking differences in the specific degree to which they become dispersed from this point. In *T. torosus* the migration of these cells is evidently very limited, with

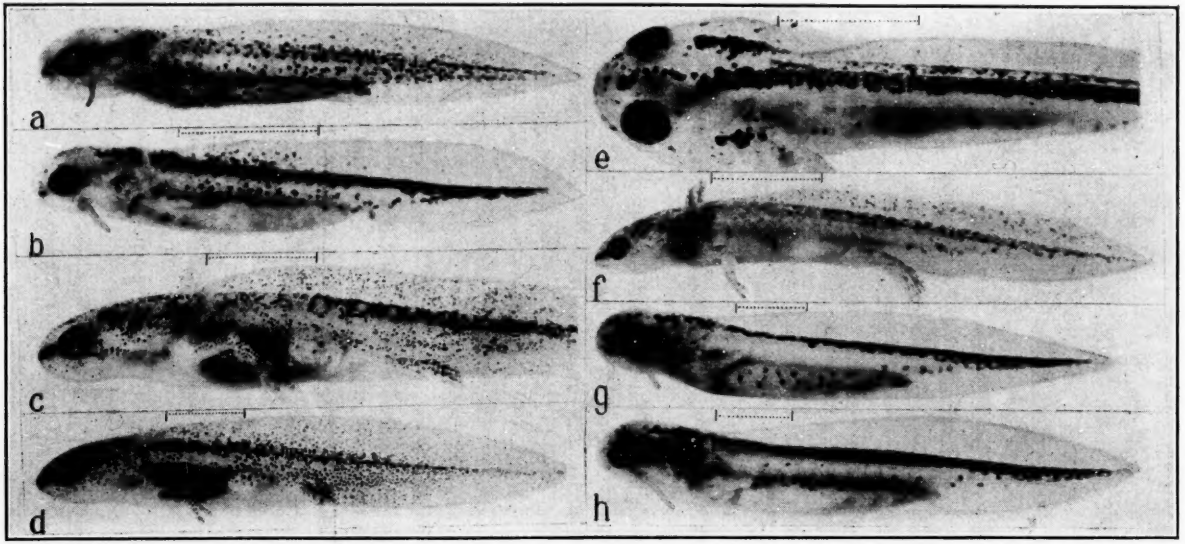

FIG. 2. *a*, young *similans* ♀ × *rivularis* ♂ hybrid, shortly after first appearance of melanophores (control for comparison with *b*). *b*, transplantation of neural crest from an embryo of *a* (*similans* ♀ × *rivularis* ♂) to a host of a third species, *T. torosus*. Note that the grafted pigment cells reproduce essentially the donor type of pattern at the level of the graft. The only important deviation is found in the failure of the grafted melanophores to descend below the yolk margin of the host. *c*, host, *torosus* ♀ × *similans* ♂ hybrid; transplant, crest cells from the paternal species, *similans*. *d*, reciprocal of *c*. Note that in both *b* and *c* the grafted pigment cells tend to retain their original prospective arrangement. *e*, host, young larva of *T. torosus;* transplant, neural crest from *torosus* ♀ × *similans* ♂ hybrid. Photographed at the beginning of independent feeding. Prior to this time the transplanted pigment bands were scarcely distinguishable from those of the host. Note, however, that the paternal influence is now becoming evident, in the disruption of the band at the level of the transplant. This change occurs at the same time as in donor controls. *f*, photograph of a similar case some weeks later, shortly before the onset of metamorphosis. The dispersion of pigment cells at the level of the transplant is now complete, again corresponding to the condition in donor controls of this age. *g*, *h*, illustrating the influence of hybridization on the yolk 'barrier' (for explanation see text). Dotted lines above each animal show extent of graft.

the result that most of the melanophores are confined to dense aggregations immediately on either side of the neural tube, in the form of paired bands along the dorsal margins of the somites. Since this strong affinity of the melanophores for one another and for any adjacent structure was so pronounced, both in normal development and under experimental conditions, it was suggested that the character of the pigment pattern in *torosus* might be attributable to marked adhesive or glutinous properties of the pigment-forming cells in this species. In the other members of the *Triturus* series, where as we have seen the melanophores gradually develop an increased freedom of movement, one might propose that the glutinous character of the pigment-forming cells is correspondingly decreased.

In connection with this hypothesis, attention was called to differences in the amount of melanin in the pigment cells of the various species, which seemed in turn to be correlated with the type of pattern formed by the cells, *i.e.,* with their degree of dispersion. The density of melanin seems in general to be especially high in melanophores of *torosus,* and lowest in those of *rivularis.* There is at present no basis for assuming a causal relationship between the quantity of contained pigment and some property akin to glutinosity, but it appears nevertheless that a rough proportionality does exist. In the present paper illustrations are provided in Fig. 2. When *torosus* eggs are fertilized by *similans* sperm, the paternal influence results ultimately in a disruption of the dorsal pigment bands. (Twitty, 1936, p. 248 and Fig. 2.) Moreover, this effect is repeated when the hybrid crest is grafted back to embryos of the maternal species, *torosus* (Fig. 2, *e, f*). Experiments of this nature provide an excellent opportunity to compare the pigmentation of the normal host melanophores with that of the transplanted hybrid cells. In young stages the host melanophores are so densely crowded into the dorsal bands that their boundaries can not be distinguished (Fig. 2 *e*), but later they

become sufficiently disengaged that one can compare them directly alongside the hybrid melanophores situated in the graft region. It is found that the visible bulk of melanin is on the average decidedly smaller in the hybrid melanophores. Thus we see that the increased dispersion of the hybrid melanophores is actually associated with an apparent reduction in their melanin content. The correlation is equally obvious when the hybrid crest is transplanted to hosts of the *paternal* species, *T. similans* (Fig. 2 *d*). Here again we see that the more heavily pigmented cells are also the ones which are more closely aggregated. The same comparison is evident in the reciprocal transplantation of *similans* crest to a hybrid host (Fig. 2 *c*).

(2) From the foregoing one may conclude that the pattern of melanophore distribution is indeed determined primarily by properties intrinsic to the pigment cells, and that it is in turn these cells which are most critically affected by hybridization. The object of the present section is to deal with an exception to this generalization which, although of secondary importance, is none the less definite. We refer particularly to factors which check the ventral migration of melanophores beyond certain limits. It was explained that in embryos of *T. torosus* the few melanophores which lie outside the paired bands never descend below the dorsal margin of the yolk mass. In *T. similans,* on the contrary, this "barrier" does not exist, and melanophores are abundantly represented on the sides of the yolk. The same is true to a lesser extent of *T. rivularis* and some of the geographical variants of *T. torosus* (Fig. 1). It was shown in the earlier publication that these differences are not based on properties of the pigment-forming cells themselves, but by the prescriptions of the host, and this has since been confirmed by many additional observations. Thus when neural crest is grafted from *similans* to *torosus,* the melanophores which descend from the transplant are halted when they first reach the yolk mass.

It was of interest to observe the influence of hybridization on this phenomenon. Since we have just seen that the

genetic nature of the pigment cells played no rôle in the original results, it was not surprising to find that the barrier itself is the component altered by the foreign sperm. A case in point is provided by experiments with *torosus-similans* hybrids. When a *torosus* egg is fertilized by sperm of *similans,* the paternal influence later causes melanophores to appear below the dorsal margin of the yolk on the sides of the hybrid. If now the neural crest of such an embryo has been replaced earlier by crest from normal *torosus,* the melanophores which descend from the transplant also come to lie on the sides of the yolk (Fig. 2 *g*). Conversely, when the hybrid crest is transplanted to *torosus,* the melanophores in question stop at the dorsal boundary of the yolk mass (Fig. 2 *h*), again conforming in this respect to the stipulations of the host instead of to their original destiny. Thus it is clear that the paternal influence on the distribution of these cells is mediated through changes in the conditions at the yolk boundary and not by any effect on the melanophores themselves. Although the nature of the factors involved is not yet clear, these experiments confirm the earlier evidence as to their localization, and extend the analysis of nuclear influence to another feature of pigment development in hybrids.

Lateral-line organs: Attention has already been called to the small unpigmented areas around the lateral line organs in older larvae of *similans* (Fig. 1, *d'*), and experiments were proposed to test the factors responsible for this distinctive characteristic (Twitty, 1936). Although no crucial evidence is yet available, one preliminary finding of some interest may be recorded at this time. It was found, namely, that when the lateral-line organs are prevented from developing by removal of their anlage in the embryo, the unpigmented areas in question fail to appear, with the result that the side of the larva presents a uniformly pigmented expanse. With this knowledge at hand, it will now be of interest to exchange the lateral-line rudiments between *similans* and other forms of *Triturus,*

in order to determine whether the differential lies in the lateral line organs themselves or in the melanophores of the various species. The same type of experiment might also be performed using hybrid embryos, in connection with the fact that the unpigmented areas become blurred when *similans* is hybridized with *rivularis*.

(3) In the foregoing we have been concerned solely with the factors which determine the *distribution* of the pigment cells. As pointed out earlier, however, there are also specific differences in the *number* of melanophores. Of the species used, *T. similans* is probably the most heavily pigmented in this respect. When we approach the question of melanophore number it becomes pertinent to examine the rôle of the ectoderm, since, as already cited, Harrison and DuShane have shown that this tissue is instrumental in stimulating the development of pigment cells. The writer found also in this connection that the differences in number of melanophores in *torosus* and *similans* are partly attributable to factors in the ectoderm. Skin of *torosus* grafted to *similans* resulted in the formation of fewer pigment cells under the transplant, whereas their number was increased by the reciprocal transplantation. For the purposes of the present study, therefore, it is in order to test the influence of hybridization on the capacity of ectoderm to elicit melanin formation. To date this attempt has been confined principally to experiments with *torosus-similans* hybrids. Fertilization of *torosus* eggs by *similans* sperm substantially increases the number of melanophores which later appears on the sides. To ascertain whether this change reflects a modification of the ectoderm by the male factors, the skin was removed from the flank of such hybrids, in young tail-bud stages, and replaced by similar ectoderm from normal *torosus* embryos. All the results agreed in showing that the original hybrid ectoderm possessed a higher capacity for provoking melanophore development than the homozygous *torosus* tissue grafted in substitution. The area covered by the transplant always contained decidedly

fewer melanophores than either the adjacent regions or the corresponding control area on the opposite side of the host. If we interpret these results in the terms suggested tentatively by DuShane (1935), we might conclude that the male influence in hybridization has increased the quantity or activity of the chemical substances delivered by the ectoderm to underlying pigment cells, and essential to the synthesis of melanin.

II. Experiments on the Development of the Dorsal Fin

A large number of transplantations, performed in a variety of connections, have shown that in *T. torosus* the trunk fin fails to develop in the region where the original ectoderm has been replaced in tail-bud stages by ectoderm from the side of the embryo. Apparently the transplanted flank skin has either lost its capacity for responding to the influences which normally elicit fin development, or these influences in question are no longer operative. This type of experiment has since been extended, and although the study is still incomplete in many respects, certain results of considerable interest have already been noted. Chief among these is the outcome of transplantations following hybridization.

Using embryos of the same developmental stage as before, the prospective fin ectoderm was removed from *torosus* ♀ × *similans* ♂ hybrids and replaced by flank ectoderm from *torosus*. In ten cases out of a total of fourteen a fin was formed from the grafted skin. In most cases these induced fins were virtually normal in size and form, and in none was there any doubt concerning their identity as fin structures. Of the remaining four cases, one was debatable, and the other three negative. Although it is planned to extend this series by further experiments, there seems to be no question concerning its significance when compared with the negative results noted above for the original experiments with *torosus*.

In view of the fact that identical tissue was grafted in both experiments, the difference in results must be attrib-

utable solely to the factors introduced into the hosts of the second series by the foreign *similans* sperm. This is of some interest, since it indicates that processes of induction are subject to hereditary change. Most of the evidence on this point might prepare one to expect the contrary. Practically all the results of hetero- and xenoplastic transplantation suggest in fact that the fundamental organizing agencies are essentially uniform in nature even in embryos of distantly related amphibian types. The distinctive features of differentiation are thus based on the specificity of reaction to these inductive stimuli. Accordingly one would not expect the latter to be modified by hybridization, and in general this would undoubtedly prove to be the case. Even in the present instance it is improbable that the qualitative character of the fin-evoking factors has been altered. It is more likely, *a priori,* that they have merely been intensified or that the period during which they operate has been shifted into a later stage of development.

It will be interesting to determine whether this change introduced by the *similans* sperm is a direct reflection of conditions in the paternal species. The meager evidence at hand indicates that this may prove to be the case. Thus a dorsal fin was formed in five out of seven cases when *torosus* flank ectoderm was grafted to the back of *similans* embryos, suggesting that the stimuli which induce fin development are indeed more active in *similans* at this stage than in *torosus.* An additional series of experiments seems to show, on the other hand, that the responsiveness of the ectoderm is *less* in *similans* than in *torosus.* When *similans* flank ectoderm was grafted in the appropriate position on embryos of the same species, a fin was formed in only one case out of eleven, with a second case debatable. The results were entirely negative when *similans* ectoderm was grafted to hosts of *torosus.* It is unnecessary at this time to describe the few additional combinations tested in this connection, since the entire question is to be reinvestigated later on a larger scale permitting statistical comparisons.

To my knowledge the possible existence of specific differences in the intensity of organizing stimuli, or in the developmental stages at which they are most active, has never been adequately investigated. An interesting situation with a possible bearing on this question is provided by studies on the factors determining direction of ciliary beat in the embryonic ectoderm. The results of Woerdemann (1923, 1925) and Holtfreter (1933) indicate that in *Rana* and *Triton* the polarity of ciliary action is fixed by the "Unterlagerung" during gastrulation, whereas in *Amblystoma punctatum* the ectoderm does not become polarized in this respect until near the end of neurulation (Twitty, 1928). Until the basis for this marked contrast has been more fully studied, one does not seem justified in attributing it solely to differences in the reactivity of the ectoderm in the species involved.

Conclusion

Since the article itself is essentially a summarized version, we may conclude with the addition of only a few general remarks.

From the experiments outlined in sections 1, 2 and 3 it is seen that in hybridization the sperm influence impinges on various components of the complex involved in pigment development. Although the most important changes are produced in the pigment-forming cells themselves, certain secondary features of their distribution, as well as the total number formed, are subject to paternal effects on their organic environment. In the absence of genetic evidence we can only conjecture whether separate gene factors are responsible for these various changes—in the melanophores themselves, in the conditions at the yolk border and in the ectodermal properties which regulate the number of melanophores formed. Hamburger (1936) has raised a similar question in connection with the phases of limb development in *Triton* hybrids, but in both instances the answer is dependent on the possibility of

obtaining successive generations from the original F_1 hybrids.

The participation of paternal and maternal factors in the effects produced appears to be approximately equal, although no precise discrimination on this point has been attempted. Thus the degree of melanophore aggregation or dispersion, the distance to which these cells may descend below the yolk border, and the numbers of pigment cells as well, are generally effected on the basis of compromise.

The final section of the paper deals with an entirely different phase of hybrid development, namely, the formation of the tail-fin. By showing that not only definitive species characters, but also the underlying processes of embryonic organization are subject to hereditary modifications, a means is opened for the study of these "inductive" influences along new lines.

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THE INHERITANCE OF THE MUTATION "PEARL" IN THE FLOUR BEETLE, *TRIBOLIUM CASTANEUM* HERBST

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IN an examination of stock cultures of the red-rust flour beetle, *Tribolium castaneum*[1] Herbst, in connection with experimental population studies, a certain individual was found which possessed eyes completely lacking black pigment in certain ommatidia. The eyes of this form appeared white or pearly when contrasted with the eye of a normal *Tribolium,* which is entirely black. This suggested at once that the unusual eye pattern might be due to some kind of heritable variation, since the failure of pigment to develop seems to be an effect quite commonly produced by gene mutation. In order to study this character genetically, a number of pupae were segregated from the stock culture and sexed (for technique see Park, 1935) and then set aside and kept in a virginal condition until examined. Out of this group of sexed pupae one pearl eyed male and one pearl eyed female were found which appeared exactly, with respect to eye pigmentation, like the original pearl eyed beetle. These two pearl forms were mated together and their progeny studied. The progeny were all pearl eyed like their parents and when bred with themselves for the purpose of multiplying the pearl stock gave all pearl eyed forms. It seemed patent, on the basis of these results, that this eye abnormality was an inherited characteristic and certain genetic crosses were made to determine the mode of inheritance of the mutation which is, to the best knowledge of the

[1] This beetle will be better known to most entomologists by the name of *Tribolium ferrugineum* Fabr. Since Good (1936) has recently shown, however, that the species is more correctly designated *T. castaneum* I hasten to comply with this change in terminology.

author, the first to be described for the family Tenebrionidae.

In all the individuals examined the pearl eye appeared the same. There was no indication of intermediacy or blending inheritance: the beetles were phenotypically either normal (*i.e.*, black) eyed or else pearl eyed like the original parents. In the pearl eye the pigment is absent from the central facets which, as a result, appear clear, while the peripheral facets are black like those of a normal-eyed beetle. Fig. 1, comparing the eyes of normal and pearl forms, brings out these distinctions. Histological preparations of the pearl eyes have not as yet been made, so that it is impossible to state whether the entire eye structure is atypical or whether, as appears to be the case, just the pigment is lacking from the abnormal eyes. Lack of this information, however, has not seemed of primary importance in studying the inheritance mechanism of the mutation.

In certain of the earlier genetic crosses two types of matings were made: mass matings, in which five males and five females were introduced initially to their respective bottle, and single matings, in which one male and one female were introduced initially. Each cross, regardless of type, was started in a bottle containing 32 gm of sifted whole wheat flour and maintained in darkened incubators at a temperature of 30° C. and at a stable humidity of around 40 per cent. The pearl-eyed stock all came, as previously described, from the one pair isolated from the stock culture. The normal *Tribolium* were taken from cultures which had been maintained in the laboratory for several years. Originally, this normal stock and the stock in which the pearl eyed beetles were discovered came from the same source. This is an important point to keep in mind, since it helps to explain several discrepancies which will be mentioned later. Each normal beetle used in a genetic cross was examined under the binocular microscope before introduction into the experimental bottle

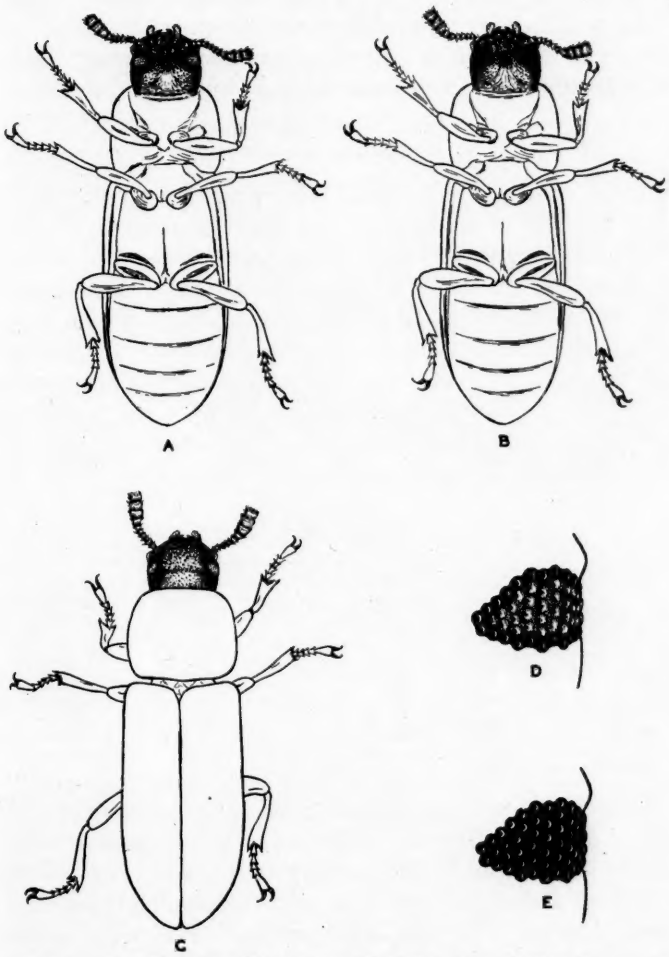

FIG. 1. Drawings from life of Normal and Pearl eyed *Tribolium castaneum*. A, ventral view of pearl eye beetle; B, ventral view of normal eye beetle; C, dorsal view of pearl eye beetle; D, enlarged view of pearl eye (ventral); E, enlarged view of normal eye (ventral). (Drawings by Mr. Arthur Johansen.)

to be sure that its eyes were phenotypically black. The same precaution was followed for the pearl beetles.

The first crosses to be made were those characteristic of a Mendelian P_1 cross and were as follows:

(1) Normal male × Pearl female
(2) Pearl male × Normal female

For each cross, thirty bottles were prepared as described with five bottles started as mass matings and twenty-five bottles as single matings. When pupae appeared in the cultures they were sifted out from the flour, sexed and put aside until they had emerged as adults, at which time they were all examined under a binocular dissecting microscope for eye color. This method of isolating pupae according to sex assures that virgin forms will be obtained for use in future crosses. A summary of the results of this first cross and its reciprocal appears in Table I,

TABLE I
THE F_1 CROSS. NORMAL × PEARL AND RECIPROCAL

Cross made	Number of normal eye progeny Pp			Number of pearl eye progeny pp			Number of productive bottles
	♂♂	♀♀	Both	♂♂	♀♀	Both	
Normal ♂ × Pearl ♀ (PP × pp)	1,301	1,353	2,654	0	0	0	28
Pearl ♂ × Normal ♀ (pp × PP)	526	632	1,158	0	0	0	25
Sum of both	1,827	1,985	3,812	0	0	0	53

Probability of the sex ratio deviating from a 1–1 ratio, = 2.5 × S.E.

where the progeny are described relative to eye color and sex. From the genetic view-point these results seem clear-cut, since all the F_1 progeny are normal eyed. This suggests that normal or black eye is completely dominant over pearl. The sex ratio deviates somewhat more than might be expected on a chance basis, there being an excess of females. The summed productivity between the two crosses is interesting and puzzling. The normal male × pearl female bottles produced over twice as many offspring as did the reciprocal bottles. The suggestion of a differential productivity appears in several places in

this report and, at the moment, it is not clear as to whether it has a genetic or an ecological basis. It does not, however, offer great difficulties in the interpretation of the data when the emphasis is restricted to the inheritance of the eye abnormality.

Using some of the F_1 progeny discussed in Table I and virgin, homozygous normal and pearl beetles from the stock cultures, the following crosses were made:

(3) F_1 normal × F_1 normal (F_2 cross)
(4) F_1 normal male × Pearl, stock female (the pearl backcross)
(5) F_1 normal female × Pearl, stock male
(6) F_1 normal male × Normal, stock female (the normal backcross)
(7) F_1 normal female × Normal, stock male

The results of the F_2 cross (No. 3), started initially as 50 single mating bottles and 10 mass mating bottles, are summarized in Table II. Here, evidence is presented

TABLE II
THE F_2 CROSS. $F_1 \times F_1$

Cross made	Number of normal eye progeny			Number of pearl eye progeny			Number of productive bottles
	♂♂	♀♀	Both	♂♂	♀♀	Both	
F_1 ♂ × F_1 ♀ (Pp × Pp)	2,562	2,611	5,173	716	770	1,486	55
Observed Ratio (per cent.)	78.1	77.2		21.9	22.8		
Expected Ratio (per cent.)	75.0	75.0		25.0	25.0		

Probability of the normal and pearl progeny deviating from a 3–1 ratio, = 5.0 × S.E.
Probability of the sex ratio of normal beetles deviating from a 1–1 ratio, = 0.6 × S.E.
Probability of the sex ratio of pearl beetles deviating from a 1–1 ratio, = 1.4 × S.E.

of genetic segregation of the pearl eye character, for, having started with normal eyed, heterozygous parents, a certain number of pearl eyed progeny are recovered in the resulting generation. Allowing P to represent normal, black eyes and p pearl eyes this cross with its expected offspring could be symbolized as follows:

$$Pp \times Pp = 1\ PP, 2\ Pp, 1\ pp$$

Here the Pp would appear normal, since normal is dominant and a ratio of 3 to 1 would result. As an actual

fact, there is a preponderance of the normal *Tribolium* with the ratio being nearer to 3½ to 1 than it is to 3 to 1. The numbers are large enough to have considerable statistical reliability, so that this unexpected ratio can not be glossed over too easily as a sampling aberration. On the basis of results to follow, however, it seems possible that pearl eye may be due to a single recessive gene. This would mean that the complication of the F_2 ratio, if not due to chance, has its origin in some secondary type of effect such as a reduction in vigor of the pearl forms under certain conditions. It is to be hoped that future work will clear up this point. As in the F_1 cross there are more females than males, although here the differences are not statistically significant. It is instructive to note that there is no indication that sex is associated in any way with the inheritance of the pearl eye, since both the males and females exhibit the character.

The results of the pearl or recessive backcross (Nos. 4 and 5) confirm the view that pearl eye is inherited as a Mendelian recessive character. Here, the F_1 normal heterozygotes were bred with homozygous pearl eyed beetles taken from the stock culture and their progeny studied and sorted. Sixty bottles in all were started with 30, five of which were mass matings, constituting the F_1 male $\times$ pearl female cross, and the other 30 constituting the reciprocal cross. The expected result of such a cross, assuming pearl-eye to be due to a single recessive gene, would be for one half of the progeny to be normal and one half pearl eyed. This could be symbolized as follows:

$$Pp \times pp = \tfrac{1}{2}\ Pp,\ \tfrac{1}{2}\ pp.$$

The actual results of this cross, expressed in Table III, confirm these theoretical expectations, for of the 2,482 progeny examined, 1,254 were normal eyed and 1,228 were pearl eyed. This difference between the two types is quite small and of no statistical significance. Furthermore, it should be emphasized that there is no evidence

that pearl eye is sex-linked, since it appeared equally distributed among both sexes in both crosses. As previously, the females in both pearl and normal groups are

TABLE III
THE PEARL BACKCROSS

Cross made	Number of normal eye progeny			Number of pearl eye progeny			Number of productive bottles
	♂♂	♀♀	Both	♂♂	♀♀	Both	
F_1 ♂ × Pearl ♀ (Pp × pp)	110	92	202	89	107	196	26
Observed Ratio (per cent.)	55.2	46.3		44.8	53.7		
Expected Ratio (per cent.)	50.0	50.0		50.0	50.0		
F_1 ♀ × Pearl ♂	397	655	1,052	412	620	1,032	27
Observed Ratio (per cent.)	49.1	51.3		50.9	48.7		
Expected Ratio (per cent.)	50.0	50.0		50.0	50.0		
Sum of both	507	747	1,254	501	727	1,228	53
Observed Ratio (per cent.)	50.2	50.6		49.8	49.4		
Expected Ratio (per cent.)	50.0	50.0		50.0	50.0		

Probability of (summed) normal and pearl progeny deviating from a 1–1 ratio, = 0.54 × S.E.
Probability of the (summed) sex ratio of normal progeny deviating from a 1–1 ratio, = 6.8 × S.E.
Probability of the (summed) sex ratio of pearl progeny deviating from a 1–1 ratio, = 6.4 × S.E.

more numerous than are the males: this is a statistically significant difference. Probably the sex ratio for the normal eyed progeny of the F_1 female × pearl male is the most unusual abnormality in the entire results. Here the deviation is so great from an expected 50–50 frequency as to suggest a sex-linked lethal effect, although no direct evidence of this in previous or later crosses is apparent.

The results of the normal backcross, made up as regards initial matings and bottle numbers precisely like the

TABLE IV
THE NORMAL BACKCROSS

Cross made	Number of normal eye progeny			Number of pearl eye progeny			Number of productive bottles
	♂♂	♀♀	Both	♂♂	♀♀	Both	
F_1 ♂ × Normal ♀ (pp × PP)	1,065	1,113	2,178	0	0	0	26
F_1 ♀ × Normal ♂	1,607	1,539	3,146	0	0	0	28
Sum of both	2,672	2,652	5,324	0	0	0	54

Probability of the (summed) sex ratio deviating from a 1–1 ratio, = 0.2 × S.E.

pearl backcross, are summarized in Table IV. On the assumption that pearl is due to a single recessive gene it would be anticipated that in a mating of a heterozygous, normal F_1 *Tribolium* with a homozygous normal beetle only normal progeny would result. This cross can be symbolized,

$$Pp \times PP = \tfrac{1}{2}\ PP,\ \tfrac{1}{2}\ Pp \text{ (all appearing black)}$$

In practically all the bottles this expectation was realized, as can be seen from Table IV where no pearl eye progeny are reported and the sex ratios are not abnormal. In three bottles (two from the F_1 male $\times$ normal female and one from its reciprocal), however, not included in Table IV, pearl progeny were produced in the ratio typical of an F_2 cross with about three normal *Tribolium* to each pearl. It seems highly probable on the basis of the previous results that in these cases the stock, normal beetles, although phenotypically normal eyed, were in reality heterozygotes, so that the actual crosses made in these bottles were $Pp \times Pp$. These results strongly suggest that the pearl mutation arose in the stocks a considerable period ago, since the normal culture used in these experiments originally came from the same source as did the culture in which the original pearl eyed *Tribolium* was first noticed. It also seems patent that the normal stocks are carrying along a small proportion of beetles heterozygous with respect to the eye characteristic and that several of these were used in making up the normal backcross series.

As Wright (1934) has pointed out, it is essential to carry a genetic experiment through some kind of an F_3 generation before critical evidence is obtained to the effect that the F_2 and first backcross ratios, although giving expected results, are actually due to a single recessive gene and not to some more complicated array. To satisfy this requirement a number of F_3 crosses were made, using the progeny of various preceding matings.

In the first group of F_3 crosses, phenotypically normal eyed beetles representing some of the progeny of the F_2

cross were mated to pearl eyed forms. These normal parents could be either homozygous or heterozygous and their progeny, depending upon the parental genetic constitution, either ½ normal and ½ pearl or all normal. The possibilities can be symbolized as follows:

(8) PP × pp = Pp (all normal)
(9) Pp × pp = ½ Pp, ½ pp

These bottles were all made up as single reciprocal matings in smaller numbers than the preceding crosses, and the appearance of the offspring are summarized in Table V. In the first part of the table the results typical

TABLE V

AN F_2 CROSS. F_2 NORMAL BEETLES (PP OR Pp) × PEARL

Cross made	Number of normal eye progeny			Number of pearl eye progeny			Number of productive bottles
	♂♂	♀♀	Both	♂♂	♀♀	Both	
F_2 N-eye ♂ × Pearl ♀ (Pp × pp)	52	41	93	42	64	106	10
F_2 N-eye ♀ × Pearl ♂ (Pp × pp)	206	231	437	216	212	428	10
Sum of both	258	272	530	258	276	534	20
F_2 N-eye homozygous ♂ × Pearl ♀ (PP × pp)	4	3	7	0	0	0	1
F_2 N-eye homozygous ♀ × Pearl ♂ (PP × pp)	33	25	58	0	0	0	1
Sum of both	37	28	65				

Probability of the (summed) normal and pearl progeny deviating from a 1–1 ratio, = 0.1 × S.E.

of a backcross are recorded. Here, the F_2 parents were obviously heterozygous for, of their offspring, about half were normal and half were pearl eyed and the expectations of mating No. 9 are realized. In the latter part of Table V two crosses are summarized which most likely represent mating No. 8, where the normal parents are homozygous. It is exceedingly curious that only two crosses out of a possible 22 were found for this type when the probability is that of the F_2 normal beetles one third should be homozygous for normal eye color. In other words, six or seven matings of the latter type instead of only two would be expected on the basis of chance. No

explanation of this aberration other than that of unusual chance deviation from the expected can be offered.

The second group of F_3 crosses was made by selecting virgin, pearl-eyed *Tribolium* from the F_2 progeny and mating them with other pearl beetles from the stock cultures. It is obvious, of course, that a mating of this type should give only pearl progeny since pearl is a recessive character and therefore must be homozygous to be phenotypically visible. This cross, made up as five single mating bottles, can be represented as follows:

$$(10)\quad pp \times pp = pp$$

The data are summarized in Table VI, from which it is apparent that only pearl eyed forms result.

TABLE VI

AN F_3 CROSS. F_2 PEARL BEETLES × PEARL (STOCK)

Cross made	Number of normal eye progeny			Number of pearl eye progeny			Number of productive bottles
	♂♂	♀♀	Both	♂♂	♀♀	Both	
F_2 Pearl ♂ × Stock Pearl ♀ (pp × pp)	0	0	0	19	17	36	3
Reciprocal (pp × pp)	0	0	0	11	8	19	2
Sum of both	0	0	0	30	25	55	5

Another F_3 cross was made where the normal eyed progeny from the first pearl backcross were again crossed with pearl eyed stock beetles. Under Mendelian expectations these normal parents should be heterozygous and the cross should therefore give a 50–50 ratio characteristic of the backcross: *i.e.*, the mating should be,

$$(11)\quad Pp \times pp = \tfrac{1}{2}\ Pp,\ \tfrac{1}{2}\ pp$$

The results of Table VII confirm this expectation, since both normal and pearl progeny appear in equal numbers.

A contrast to the preceding F_3 cross is seen when the pearl eyed beetles from the first pearl backcross are mated with pearl forms from stock cultures. The expectations here are, of course, similar to those of cross 10, although the experimental parents have a different

TABLE VII

AN F_2 CROSS. NORMAL BEETLES FROM THE PEARL BACKCROSS × PEARL STOCK

Cross made	Number normal eyes ♂♂	♀♀	Both	Number pearl eyes ♂♂	♀♀	Both	Number of productive bottles
PBC N ♂ × Pearl ♀ (Pp × pp)	25	31	56	27	28	55	5
PBC N ♀ × Pearl ♂ (Pp × pp)	21	26	47	31	18	49	7
Sum of both	46	57	103	58	46	104	12

Probability of (summed) normal and pearl progeny deviating from a 1–1 ratio, = 0.6 × S.E.

genetic history. The results, reported in Table VIII, confirm the fact that pearl eyed *Tribolium* always breed true when mated with themselves, since no normal forms appeared among the offspring.

TABLE VIII

AN F_2 CROSS. PEARL BEETLES FROM THE PEARL BACKCROSS × PEARL STOCK

Cross made	Number normal eyes ♂♂	♀♀	Both	Number pearl eyes ♂♂	♀♀	Both	Number of productive bottles
PBX Pearl ♂ × Pearl ♀ (pp × pp)	0	0	0	18	17	35	3
Reciprocal (pp × pp)	0	0	0	5	4	9	1
Sum of both	0	0	0	23	21	44	4

In addition to using beetles from the F_2 and pearl backcross experiments in F_3 matings, *Tribolium* from the normal backcross progeny were also mated as a final check on the segregation of pearl eye in the third generation. These crosses were similar to those made earlier (Nos. 8 and 9) in which normal-eyed beetles, from the first normal backcross, were crossed in reciprocal with pearl-eyed forms. As before, the possibilities are that the normals may be either heterozygous or homozygous with the expectation that half of them will be PP and half Pp. The crosses made here may be either of the following types:

(12) $Pp \times pp = \frac{1}{2}\ Pp, \frac{1}{2}\ pp$
(13) $PP \times pp = \frac{1}{2}\ PP, \frac{1}{2}\ Pp$ (all normal)

The results, tabulated in Table IX, show that more heterozygous normal beetles were selected than were homozygous forms. The same thing was true, to a greater degree, for matings 8 and 9, although all these F_2 crosses gave expected results as far as the segregation of pearl eye was concerned. In the upper part of Table IX, six

TABLE IX

AN F_2 CROSS. NORMAL BEETLES FROM THE NORMAL BACKCROSS × PEARL STOCK

Cross made	Number normal eyes			Number pearl eyes			Number of productive bottles
	♂♂	♀♀	Both	♂♂	♀♀	Both	
NBX Normal Heterozygous ♂ × Pearl ♀ (Pp × pp)	37	23	60	33	44	77	4
NBX Normal Heterozygous ♀ × Pearl ♂ (Pp × pp)	25	22	47	17	20	37	2
Sum of both	62	45	107	50	64	114	6
NBX Normal Homozygous ♂ × Pearl ♀ (PP × pp)	None						
NBX Normal Homozygous ♀ × Pearl ♂ (PP × pp)	109	91	200	0	0	0	2

Probability of (summed) normal and pearl progeny deviating from a 1–1 ratio, = 0.4 × S.E.

separate matings of a heterozygote normal beetle to a pearl form (Cross 12) are recorded. The expectations are that half of the progeny will show normal eyes and half pearl eyes. This is confirmed by the data, since out of 221 *Tribolium* recorded, 107 were normal and 114 pearl, with no statistical differences between the two types. The sex ratios show no significant deviation from an expected 1 to 1 ratio. In the lower part of the table two crosses of homozygous normal beetles by pearl forms (Cross 13) are summarized. Here, as would be anticipated, only normal eyed forms appear and no important deviations in the sex ratio are apparent.

CONCLUSIONS AND SUMMARY

On the basis of various genetic matings reported in this paper a new mutation in the flour beetle, *Tribolium castaneum* Herbst, has been described. The mutation, designated "pearl," when present in a homozygous con-

dition causes the eyes of the beetles to lack black pigmentation in certain of the central ommatidia. This is in contrast to the eyes of normal or "wild" *Tribolium,* which are entirely black. Normal eyes are always completely dominant over pearl eyes, and the genetic ratios suggest that the presence of pearl eyes may be due to a single recessive gene. This is attested by the following facts: first, F_1 beetles from normal by pearl parents always have normal eyes; second, F_2 beetles from a self-mating of F_1 progeny exhibit the pearl eye in a ratio of approximately 1 pearl to 3 normal beetles; third, backcross progeny from a mating of F_1 normal beetles to pearl stock beetles are half normal eyed and half pearl eyed, and fourth, pearl beetles mated with other pearl forms always give pearl offspring. F_3 tests were also made of the F_2 and backcross progeny to be sure that pearl would segregate out in a predictable way in the third generation.

Although these points about the inheritance of the pearl mutation itself appear quite clear there exist in the data a number of discrepancies which must be mentioned here, even though no explanation for them is apparent at the moment. In the first place, the sex ratios are unusual in many of the crosses with a tendency for females to be produced in more numbers than males. This is especially true in the F_1, the F_2 and the first pearl backcross matings (see Tables I, II, III) where the difference has statistical reliability. If a total of all the beetles examined for all crosses is obtained it brings out the point that of 20,133 *Tribolium,* 9,716 were males and 10,417 were females. This is a significant deviation from an expected 1 to 1 ratio, the difference being 5 times its standard error. There is no indication, however, that the pearl eye mutation is a sex-linked character. In other of the crosses the sex ratios are quite typical (Tables IV, V, VII, VIII).

The differential productivity exhibited by certain crosses is also very puzzling. An extreme example of

this can be seen in Table III, where 26 bottles of the mating F_1 male by pearl female produced 398 offspring, while 27 bottles of the reciprocal mating, F_1 female $\times$ pearl male, produced 2,084 offspring. It is obvious that some extremely potent factor is operating in this and in related cases. There may be either a genetic or an ecological basis for the differential productivity; it is impossible to venture an explanation at this time. A future problem, however, certainly lies here.

Another discrepancy in the data lies in the fact that the F_2 ratio is closer to a $3\frac{1}{2}$ to 1 than it is to a 3 to 1 and the numbers are large enough to make the departure from the expected statistically significant. This point has already been discussed earlier in the paper and it is unnecessary to elaborate it here. Finally, the marked deficiency of homozygous normal beetles in F_3 tests of F_2 and normal BC populations should be mentioned. It is curious that only 4 such *Tribolium* were found out of a possible 30 where 11 would be expected.

It should be brought out that these various aberrations in the results have been emphasized to forestall the possible criticism that they were ignored or missed in the interpretation of the genetic data. The aberrations leave some doubt as to whether the pearl eye is actually due to a single recessive gene, as certain of the data suggest, or whether some other genetic or ecological mechanism, not apparent at the moment, is operative. That future experiments are necessary to definitely settle the matter is obvious. The chief emphasis of this paper has been on describing a new variation for *Tribolium castaneum* and pointing out that this variation is inherited.

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TRANSPLANTATION OF WING–THORACIC PRIMORDIA IN *DROSOPHILA MELANOGASTER*

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INTRODUCTION

THE new method of experimental attack on the problem of the action of specific genes recently offered by Ephrussi and Beadle (Ephrussi and Beadle, 1935a, 1935b, 1935c and 1936; Beadle and Ephrussi, 1935a, 1935b and 1936) has already been productive of much interesting and valuable data. These investigators have so far concerned themselves with studies of the homoplastic transplants of the larval eye-disks of *D. melanogaster* and with interspecific transplantations of ovaries between *D. melanogaster* and *D. simulans*. Both of these types of implanted tissues lend themselves to accurate recognition of gene action, for the matching of eye color and the determination of species of progeny after interspecific grafts offer no great difficulties in the analysis of the end results.

In this paper we present the results of transplantations of wing-thoracic disks, a study primarily undertaken to determine the experimental value of implanted wing and bristle-carrying tissues in our understanding of the mechanism of gene action.

MATERIALS AND METHODS

The method used by us differs in certain features from that described by Ephrussi and Beadle (1936). It was found that the entire operative procedure may be carried out by one person (in this case, the first author), although of necessity the rapidity with which a large number of operations may be performed is obviously greatly decreased. However, by practice, the desired degree of

etherization of hosts can be timed to coincide with the availability of freshly dissected disks for implantation. The slide to which the hosts adhere can be firmly held down on the stage of the binocular by the fourth and fifth fingers of the right hand (Fig. 1), while at the same time the thumb and the other two fingers grasp the adapter of the standard Chambers microinjection circuit. To assure a smooth operative thrust at the proper angle, the host larvae were oriented with their antero-posterior axes lengthwise of the microscope slide, their anterior ends to the left. This orientation should be carried out as soon as the larvae relax under ether and before they have been

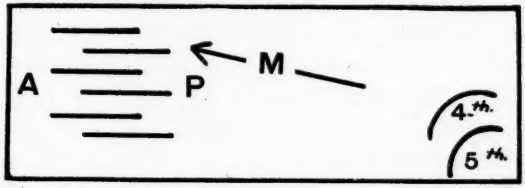

FIG. 1. A-P, orientation of larvae on operating slide. M, microneedle showing angle of thrust. Position of fourth and fifth fingers indicated at lower right of slide.

allowed to dry. A thin film of the softened top layer of agar in which the larvae have been reared serves, on drying, to hold the hosts firmly in an outstretched position. The fluid medium used was that found by Bělăr (1929) and by Baumgartner and Payne (1931) to be isotonic with the living germ cells of the grasshopper. It is prepared from stock solutions of 9 per cent. NaCl, 1 per cent. anhydrous $CaCl_2$, 1 per cent. KCl and 10 per cent. dextrose solution as follows: Combine 20 cc of the NaCl, 4 cc of the $CaCl_2$ and 5 to 10 cc of the dextrose solutions. Dilute this mixture with water to 200 cc. It is best to prepare this immediately before using, and last of all to add 0.4 cc of a 10 per cent. solution of $NaHCO_3$.

Except for a few cases, both host and implant were of approximately the same age, *i.e.*, four days ("standard" age according to Ephrussi and Beadle).

With few exceptions, the point of puncture was posterolateral, the implants being ejected from the micropipette toward the anterior end of the body cavity of the host larva. In almost all cases they were subsequently recovered from the anterior region of the abdomen of the adult. One was lodged in the muscle tissue of the thorax.

The graft disks used were the dorsal mesothoracic. The origin, shape, size, location, time of development and fate of these disks have been fully described by Chen (1929). In addition to the wings, these primordia give rise to two other adult body regions, the scutellum and the dorso-lateral portion of the thorax. They are the shape of an isosceles triangle in the normal larva. The tapering end gives rise to the thoracic regions, the wing being derived from a horse-shoe shaped ridge which arises in the posterior portion late in larval history. Because of its plasticity and shape the entire disk is easily drawn up into the micropipette (Fig. 2). However, in several cases (Table I) only a portion of the disk was implanted.

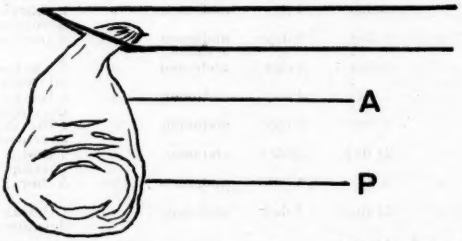

FIG. 2. Diagram to show dorsal mesothoracic disk being picked up by micropipette. A, region of scutellum and dorso-lateral thorax; P, wing region.

The stocks of wing and bristle mutants used in the test experiments were obtained from the genetics laboratory at Washington Square College, and had been carefully maintained for periods of three to five years. Those used as donors were:

(1) ey^D/ci^D, a dominant form of eyeless and cubitus interruptus. The 4th and 5th veins do not reach the wing margins. The wings are held in an outspread position due to their fusion with the allula.

(2) D/lcL, a balanced lethal stock with certain bristles, *e.g.*, presuturals, anterior dorsocentrals, missing except in very rare cases (Plunkett, 1926, and Sonnenblick, unpublished data). The wings are outspread as in ci^D.

(3) D^3, an allelomorph of Dichaete but possessing + bristles. The wings are outspread as in ci^D.

(4) H^2 ext, which appeared in an H^2 culture and evidenced the bristle-removing character in even more extreme form. It was therefore called H^2 extreme. This stock has been maintained for three years by mating brothers and sisters which presented this characteristic in the most extreme form. The H^2 ext mutant differs from scute races in the presence, at the site of each missing bristle, of a circular pit, the trichopore, bordered by a raised annulus.

Experimental Data

A record of the implantations is embodied in Table I.

TABLE I

Data on Growth and Differentiation of Mutant Dorsal Mesothoracic Implants into Wild Type Hosts

		Age of		Site of		Condition of
Implant	Host	Implant	Host	Implant	Host	Implant
7 ey^D/ci^D	+	4 day	4 day	abdomen	+	4 wing only 3 thorax and wing
3 D/lcL	+	4 day	4 day	abdomen	+	3 thorax and wing
2 D/lcL	+	3 day	4 day	abdomen	+	2 thorax and underdeveloped wing
1 D^3	+	4 day	4 day	abdomen & thorax	*	1 thorax and wing
15 D^3	+	4 day	4 day	abdomen	+	5 wing only 10 thorax and wing
1 D^3	+	3 day	4 day	abdomen	+	1 thorax and underdeveloped wing
2 D^3	+	3 day	3 day	abdomen	+	2 thorax and underdeveloped wing
1 D^3	+	2½ day	2½ day	abdomen	+	1 thorax and underdeveloped wing
5 H^2 ext	+	4 day	4 day	abdomen	+	5 thorax and wing
1 H^2 ext	+	2½ day	4 day	abdomen	+	1 thorax and underdeveloped wing

* Fly with right anterior dorso-central bristle missing. See text.

Growth and Differentiation of Implanted Dorsal Mesothoracic Disks

As previously noted, one implant was found embedded in the muscles of the host thorax. In almost all cases, however, the implants were recovered from the abdominal region of the host. In the females they were frequently found embedded between the two ovaries, a position which masked their presence to a great extent. In the males their position, size and even their color could be easily

seen through the abdominal wall. In four cases, the implant gave rise to a rough knobby external growth, smoothly attached to the abdominal wall. In two of these cases a small amount of wing tissue was identifiable.

Graft disks from 4-day larvae into hosts of the same age are found, when the hosts emerge, to have attained a size approximating the normal. Scutellar, dorso-lateral thorax and wing regions are clearly differentiated, the two thoracic portions inverted so that bristles and hairs are on the inner surface, while the wing portion, enclosed in a sac-like retaining wall, is curled tightly back on itself (See Fig. 3).

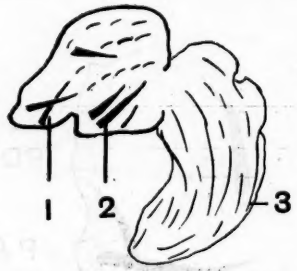

FIG. 3. Implant with well-defined scutellar (1), dorso-lateral thoracic (2), and wing (3) regions.

If the implants are removed from the host flies at once the color of the thoracic regions is found to be a yellowish brown, in sharp contrast with the gray color of the wing. The entire implant has therefore the coloring normal for similar body regions in a newly hatched fly. If, however, the graft tissue is allowed to remain for several days within the host fly, all the tissues darken, and the wing turns brown.

In disks from larvae of 2½ to 3 days transferred to 4-day hosts, growth and differentiation has, on eclosion of the host, reached a point where the three expected regions are recognizable. The two thoracic portions seem proportionately further developed than the wing region, the latter showing the characteristic gray color only at the wing base.

Well-developed implants freed from host tissue were carefully dissected open, mounted under higher powers and examined to test the possibility of identifying and distinguishing mutant characters. Although great care was taken to spread the wing tissue and to flatten it by compression under the cover-slip, in no case could the veins be recognized. Surface hairs show the usual orderly arrangement, and marginal hairs can be identified by their size and arrangement. The bristle-carrying tissues, *i.e.*, half-scutellum and half-thorax, offer a much more interesting picture. Specific bristles can be identified, and the rows of thoracic hairs stand out distinctly (Fig. 4).

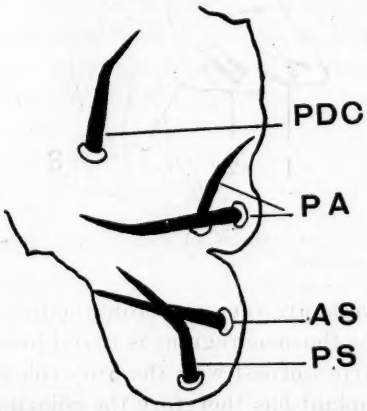

FIG. 4. Scutellum and portion of the dorso-lateral thoracic region of a *D. melanogaster* + implant. The anterior (A) and posterior (P) scutellar, the two post-alar (PA) and the posterior dorso-central (PDC) bristles can be identified by their relative position and size.

MUTANT DISKS IN WILD TYPE HOSTS

Host flies carrying well-developed implants from ey^D/ci^D show no changes in eye or bristle characteristics. The wings of the host are normal with respect to venation and flexion. Since in the implanted wing tissue no veins are recognizable, it is not possible to say whether autodifferentiation has occurred. Although no critical check

was undertaken, the scutellar and thoracic regions show hairs and bristles which appear normal in size and distribution (Fig. 4). The larger bristles may be slightly bent or curved, an effect due in all probability to the extreme compression exerted on them during their development within the inverted implant.

No host effect was observed in flies carrying D/lcL implants. In host flies with disks transplanted from D^s donors, the wing posture was normal. In 19 out of 20 successful transfers, the D^s donor tissue developed in the abdominal cavity of the host. All these flies had + bristles. In the exceptional case, the thoracic portion of the implant lodged and developed in the antero-dorsal thoracic region of the host. This fly lacked the right dorsocentral bristle, a condition observed by Plunkett (1926) and by the authors (unpublished data) to occur in both + flies and D^s rarely if ever. It is possible that this deficiency may have been caused by some mechanical injury, since the implant developed closely adpressed to the host region affected. An impracticably large number of experiments would be necessary to determine the significance of this isolated case.

Wild type flies carrying H^2 ext implants have + bristles. In the implanted disks, the H^2 ext character is autonomous in its development. On the thoracic portions of the implant few if any bristles develop, but the site of missing bristles is marked by the presence of trichopores.

DISCUSSION

The hypothesis offered by Ephrussi and Beadle to account for failure of implanted eyes to undergo autonomous development, or, in rare instances for variation from the expected eye color in hosts carrying eye implants, involves the assumption that specific "diffusible substances" given off by developing anlagen may be carried to other parts of the larva and there exert a definite effect. Assuming the presence of such a diffusible factor in our experiments, it is obvious that if such a substance

were given off from the wing-thoracic disk, it brought about no recognizable phenotypic modification of the host.

From the above data it is evident, moreover, that implanted wing tissue may not always offer a sound basis for critical interpretation of gene action. It seems improbable that, without the aid of some specially devised method, mutant characters which affect wing venation could be recognized. However, the analysis of wing dimensions in mutants which reduce wing size appears to offer greater possibilities.

On the other hand, an analysis of results on bristle-bearing tissue strongly suggests that these regions will offer reliable and accurate material for experimental interpretation. This is especially true of the scutellar region, in which, developing as it does set off from the remainder of the thorax, the number and condition of bristles may be determined with ease.

Acknowledgment

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Summary

(1) Dorsal mesothoracic disks from 4-day Drosophila larvae transplanted into host larvae of the same age grow and differentiate to form three distinct regions, *i.e.*, scutellar, dorso-lateral thorax and wing.

(2) In implants from 2½- or 3-day donors into 2½-, 3- or 4-day hosts, the wing was underdeveloped.

(3) Disks from ey^D/ci^D, D/lcL and D^3 larvae into + hosts failed to affect the venation and flexion of the host wings. In the series of D^3 implants in + hosts, all hosts carrying abdominal implants had + bristles. One host with a thoracic implant showed bristle deficiency.

(4) H^2 ext implants developed autonomously but gave no host effect.

(5) Venation defects can not be recognized in wing implants, but from the degree of growth and differentiation which the wing tissue attains it seems possible that a study of mutants which reduce wing size might yield significant data.

(6) The preliminary findings on bristle-bearing tissue strongly suggest that results on these tissues would have an experimental value comparable with that offered by eye disk transplantation.

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STUDIES ON THE LIFE CYCLE OF *CAMPELOMA RUFUM*, A FRESH-WATER SNAIL[1]

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INTRODUCTION

THE family Viviparidae is represented in North America by four genera of fresh-water, ovoviviparous snails. The type genus of the family (*Viviparus*) has very broad geographical distribution. Several of its species have been investigated and have been found to show surprising inconsistencies in details of the life cycle. *Viviparus bengalensis,* which has separate sexes, has been studied in detail in India by Annandale and Sewell (1921) and by Prashad (1928). According to Alonte (1930), *V. angularis* of the Philippines is hermaphroditic and in many other respects differs from other species of the genus. An American species, *V. contectoides,* has been investigated by Van Cleave and Lederer (1932), who have outlined the life history and have called attention to the sexual dimorphism which is associated with a differentiation in the length of life span for the two sexes.

Unlike the genus *Viviparus,* the genus *Campeloma* is exclusively North American in its distribution. Though its species are very widely distributed over eastern North America, little attention has ever been directed to the details of the life history of any species. Most of the fragmentary statements in the literature have been but incidental records, and in a number of instances unwarranted generalizations have been drawn from limited observations. It has therefore seemed desirable to make a detailed study of a member of this peculiarly American genus of snails. By mere chance the species selected for

[1] Contributions from the Zoological Laboratory of the University of Illinois No. 496.

this study (*C. rufum*) has proven to be radically different in a number of points from the fragmentary information previously accepted as distinctive for all members of the genus. In the present program of studies observations on other species have been carried far enough to discredit the early belief fostered by the morphological type concept which assumed fundamental agreement in the biology of closely related species. The detailed observations and generalizations presented in the present contribution apply to the single species, *Campeloma rufum*.

Campeloma rufum occurs in abundance in the Salt Fork River, about eighteen miles southeast of Urbana, Illinois. A small dam across the Salt Fork, in a privately owned tract of land known locally as Homer Park, helps to maintain especially favorable conditions for molluscan life for several hundred yards down stream. Through a number of years *Campeloma* has been under observation in this area and collections have been submitted to Bryant Walker, of Detroit, to H. A. Pilsbry, of the Philadelphia Academy of Natural Sciences, and to Frank C. Baker, of the University of Illinois, each of whom has identified all the shells submitted as belonging to the single species *Campeloma rufum*. Baker (1922) in a molluscan survey of the Salt Fork, had previously demonstrated the fact that this one species is the only representative of the genus *Campeloma* in the area under consideration. Consequently, here are present conditions especially favorable for conducting a biological study. In other regions, where several species of *Campeloma* occupy the same territory, considerable difficulty is experienced in segregating mixed samples, for the species of *Campeloma* are not readily distinguishable in all stages of development.

After several years of general field observation on *Campeloma rufum*, the senior author began a detailed field and laboratory program in the summer of 1930. This program of study has now extended over a period of approximately four years. From September, 1931, to June, 1932, the junior author collaborated in the investi-

gation, making intensive field studies and laboratory analyses which have been continued and extended by the senior author.

Field notes and quantitative collections have been taken at frequent intervals during the four years, but the observations have been interrupted on several occasions by stages of excessively high water, which prevented carrying out a regular, stated program of collecting. The shells of the periodic samples have been measured with a vernier caliper, and extended studies of the soft parts have been included. Methods of study were much the same as those detailed in the paper on *Viviparus contectoides* by Van Cleave and Lederer (1932).

TABLE I

SUMMARY OF FIELD COLLECTIONS OF *Campeloma rufum* FROM THE SALT FORK AT HOMER PARK, ILLINOIS

Date	Number of individuals	Graph no.
Sept. 30, '30	34	
Sept. 29, '31	166	
Oct. 25, '31	68	
Apr. 23, '32	91	
May 15, '32	78	3
June 4, '32	65	
June 25, '32	105	
Aug. 13, '32	68	5
Sept. 5, '32	207	6
Sept. 1, '33	236	
Oct. 21, '33	53	7
Jan. 13, '34	52	1
Mar. 3, '34	25	
Apr. 15, '34	146	2
May 19, '34	171	
July 3, '34	167	4
Total	1,732	

Free-living individuals of *C. rufum* in the Salt Fork vary from about 3.5 mm to 40 mm in shell height. The smallest of these were well within the limit of size attained by the uterine young. During the period of uterine development the young shells form approximately three or three and one half whorls. Adults of the species have six or seven whorls to the shell.

In the present study, attention has been directed particularly to sex, to a determination of the reproductive and growth cycles and to the uterine young.

SEX IN CAMPELOMA RUFUM

Practically every reference dealing with the biology of *Campeloma* and of the other Viviparidae gives the statement that the sexes are distinguishable on differences in the tentacles of the males (Call, 1887; Baker, 1928, Fig. 23). The only exception to the foregoing is the publication of Alonte (1930) which demonstrated hermaphroditism in *Viviparus angularis* of the Philippines. Characteristically, for all other members of the Viviparidae, it has been assumed that the condition of separate sexes, amply demonstrated for some species, maintains throughout the family. For a number of species it has been conclusively established that the right tentacle of the male is distinctly enlarged and modified as an intromittent organ, while in the female both tentacles are alike and are acutely pointed. This means of differentiating the sexes was well exemplified in the species *Viviparus contectoides,* where the sexes were readily separable even in the newly born young (Van Cleave and Lederer, 1935). Similar sexual differences have been observed repeatedly in studies on both living and preserved specimens of some species of *Campeloma* and in one species of the genus *Lioplax* (Van Cleave and Chambers, 1936). However, in *Campeloma rufum,* several thousands of specimens have been examined critically in the course of the present study without discovering a single male individual. In the early stages of this work a few individuals with blunt right tentacle were isolated as suspected males, but on autopsy every one of these possessed a uterus which, in a majority of instances, bore either eggs or young snails. In a number of cases these individuals with enlarged tentacles were dissected, but no trace of a vas deferens leading into the right tentacle could be found.

Though the literature contains numerous references to the infrequency of males in various species of the genus *Campeloma,* the complete absence of separate males in *Campeloma rufum* in the habitat under consideration

stands without precedent in the literature on this genus. A review of the literature reveals the fact that while male genitalia have been described and figured for several species of *Campeloma,* no one has previously made a detailed study of *C. rufum.* Baker (1928: 69) states of *C. rufum,* "of sixty specimens examined for genitalia, all but one were females," but he records nothing further about this one suspected male. In the same monograph (Baker, 1928: 67), he calls attention to the rareness of males in *C. integrum* and adds (p. 61) that in one hundred individuals of *C. decisum* collected in July and August but one male was found. Since Baker has shown (1928: Fig. 24) carefully detailed drawings of the male genitalia of *C. integrum,* there can be no reasonable question as to his interpretation of the sexual differences in this species. On the other hand, the present writers are inclined to believe that the asymmetry in the tentacles of the supposed male of *C. rufum* figured by Baker (1928, Fig. 23) may represent accidental injury to the tentacle, for similarly deformed tentacles were observed in the present study, though they were found to be without value as a sexual character.

Relatively large field collections, representing every season of the year, have been studied critically in the hope of discovering some seasonal factor in this problem of suppression of the males, but male individuals have been wholly lacking in every sample. In the field, every precaution was taken to avoid selection of or exclusion of any type of habitat. On the assumption that environmental factors might have caused a segregation of the sexes, every type of habitat was repeatedly examined. Finally, when prolonged field studies failed to throw any light upon the problem of sex in *C. rufum,* Mr. N. T. Mattox undertook an investigation of the problem seeking solution through the study of serial sections. The present writers are indebted to Mr. Mattox for previously unannounced results. In a paper prepared for presentation before the 1936 meeting of the American Society of Zoologists (ab-

stract in press in the *Anatomical Record*), Mr. Mattox is announcing the conclusion that *C. rufum* undergoes parthenogenetic development. Prolonged and intensive studies of serial sections prepared from material representing all seasons of the year have failed to give evidence of spermatozoa or of sperm cells in any stage of development. Cytological details of the gonad are being studied by Mr. Mattox, whose full results will be published later.

Intensive seasonal studies have eliminated the possibility of a cyclic change in sex such as occurs in protandrous organisms. The modification which has followed the elimination of separate sexes in *C. rufum* has been accompanied by profound alterations in the genitalia, for there is no vestige of an intromittent organ.

Analysis of Population Samples

The fact that separate sexes are wholly lacking eliminates one source of difficulty in the analysis of population samples of *C. rufum*. In spite of this one favorite condition, the analysis of the periodic collections failed to give such consistent pictures from season to season and from year to year as were encountered (Van Cleave and Lederer, 1932) in the study of *Viviparus contectoides*. In one series of collections taken at approximately monthly intervals, one set of specimens might show surprising inconsistencies from the general conditions revealed in other samples of the same series. Thus on May 15, 1932, rather larger numbers of recently extruded young were found. On June 4, 1932, a sample from the same locality likewise contained goodly numbers of the young which were, on the average, about one millimeter larger than those collected three weeks earlier. Fair numbers of young were likewise taken on August 13 and on September 5 of the same year, but an intervening sample on June 25 gave only one individual smaller than the 19 mm group in a total of 105 unselected specimens. This last-named sample was very obviously non-representative, for the general trend of the

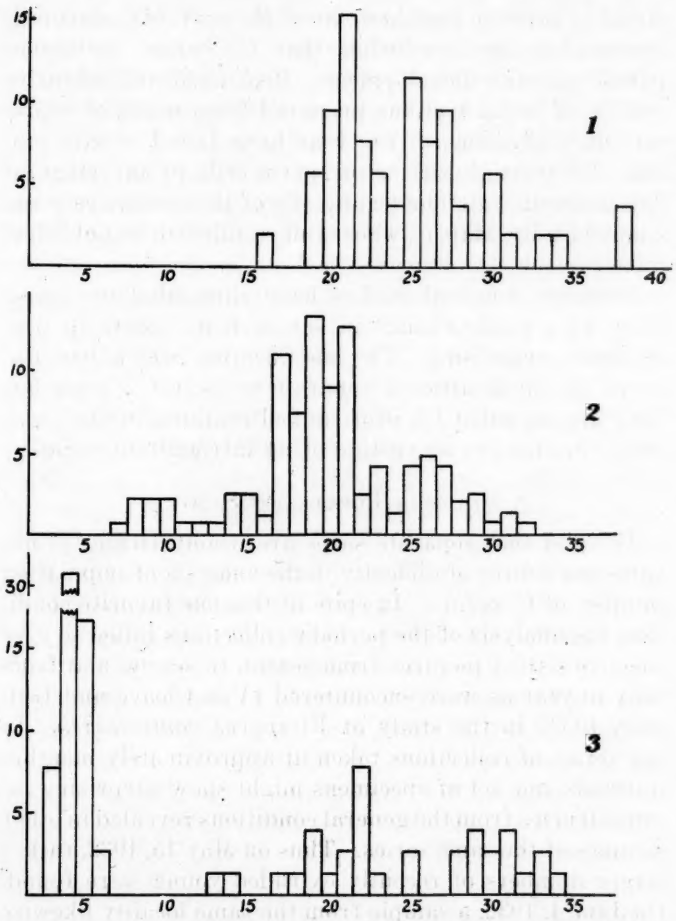

FIG. 1. A typical mid-winter sample of *Campeloma rufum*, taken on January 13, 1934. FIG. 2. A typical early spring sample taken on April 15, 1934, before the new brood had yet appeared in numbers. FIG. 3. A population sample showing the new generation at the height of the parturition period, May 15, 1932.*

* Explanation of Graphs 1–7: Each graph represents a single population sample. Size classes are based on one millimeter increments of shell height. For ease in comparison of samples, the number in each class has been reduced to percentage of the sample.

samples indicated plainly a consistent advance of the mode for the young snails except for this one date.

With a mode of shell height in the 4 mm group at birth, in May (Fig. 3), the young snails grow rapidly until the mode in September (Fig. 6) has reached the 12 mm group. This general statement is borne out by the trend of collections made in other years. Field and laboratory observations indicate that most of the individuals of the parent generation have reached a shell height of 20 mm by the time the new brood makes its appearance in May (Fig. 3). Most of the individuals below that size are without young and in a number of instances they were found to be heavily infested with trematode larvae. From data given in Table II, it is evident that the 20 mm group normally coincides with the attainment of gravidity, for this is the minimum size group in which either eggs or young are found at practically all seasons of the year. Thus *C. rufum* begins to produce at the age of approximately one year. From the 20 mm size to the maximum of 40 mm, in the habitat under consideration, *C. rufum* continues to bear young, though in some collections there is a tendency for individuals of maximum size to have empty uteri. Especially is this noticeable in the summer and fall when relatively few individuals above 30 mm in height are gravid. Most of these seem to be senescent individuals which die off during the fall and winter.

Distribution modes are not emphatically represented in the range of mature individuals, as shown by graphs 1 to 7. The only consistent mode for shell height of individuals more than one year of age is at or near the 30 mm group (Figs 1, 3, 4, 5, 6) which all available evidence seems to indicate is the mode for individuals two years of age. It thus seems evident that in the habitat under consideration *C. rufum* attains an age of two years, while possibly some individuals of maximum size represent a third annual generation.

For some of the small streams of Wisconsin, Baker (1928: 72) has recorded shells of *C. rufum* as large as

48 mm in height. If these records were authentic, it might seem that the Salt Fork does not present optimum conditions for the development of individuals of this species. However, in a personal communication Baker has informed one of us that there is reason for doubting the identification of some of these largest shells. He further states that when Bryant Walker examined specimens of *Campeloma rufum* from the Salt Fork he pronounced them the largest representatives of the species that had ever come to his attention.

Seasonal Life History

Call (1886: 165) makes the general statement for members of the genus *Campeloma,* that "During the late fall the animals bury themselves deeply in the mud, and hibernate until early spring." In the present study on *C. rufum,* no tendency toward hibernation has been apparent. Samples have been taken from the Salt Fork in every month of the winter, with no perceptible difference in activity or availability of the snails. Except for inconvenience and discomfort to the collector, mid-winter collections were readily available.

There is no season of the year when young are wholly wanting in the marsupia of the parents, but population samples taken in June gave the highest incidence of barrenness for individuals old enough to bear young. Even in the June samples some uterine young were encountered. Thus it seems evident from the examination of the marsupia that young are being engendered by at least a part of the bearing population continuously throughout the year. However, the fully formed young are not liberated at a uniform rate at all seasons. Evidences from the marsupia as well as from stream collections unite to demonstrate that the most active period of parturition comes in the spring (May). Stream collections in May and June contained the largest representatives of free young, ranging in size from 2.5 mm to 6 mm in height of shell (Fig. 3).

One series of free individuals taken on May 15, 1932 (Fig. 3), contained 45 small individuals, all of which were under 6.5 mm in shell height. Thirty of these young were under 4.5 mm high and thus were well within the limits of size attained by the young during their sojourn in the maternal uterus. In no other seasons of the year were free young secured in such abundance. Throughout the summer and until early September, a few scattered living shells under 4.5 mm were found in the population samples, but never in numbers indicating that a general liberation of young had taken place. Some of these, as in Fig. 6, might readily have been of the 3.0 mm group, liberated in May. Collections taken in late September and in October were wholly devoid of small shells comparable in size to the young at birth. In a mid-winter sample (Fig. 1) taken on January 13, 1934, the smallest free-living snail was about 16 mm high. From the foregoing it seems apparent that in *Campeloma rufum,* in the habitat under consideration, parturition is at its height in early May, continues at a diminishing rate until some time in early September, then ceases entirely during the fall and winter months.

During the fall and winter, mature individuals are engendering a new brood. Three adult specimens of *C. rufum* taken from the Salt Fork at Homer Park, Illinois, on October 25, 1931, were kept in a laboratory aquarium until April 18, 1932. Other individuals collected at the same time were examined on the day of collecting and found to be bearing large numbers of shelled young, most of which were about average size for uterine shells. The mode for height for the young examined on October 25 fell in the 3.25 to 3.34 mm group. Under conditions of the laboratory, with no attempt to simulate conditions of the natural habitat, the uterine young grew within the parents until the mode of distribution on shell height rose to the 3.75 to 3.84 mm group when the parent snails were autopsied on April 18, 1932. During this

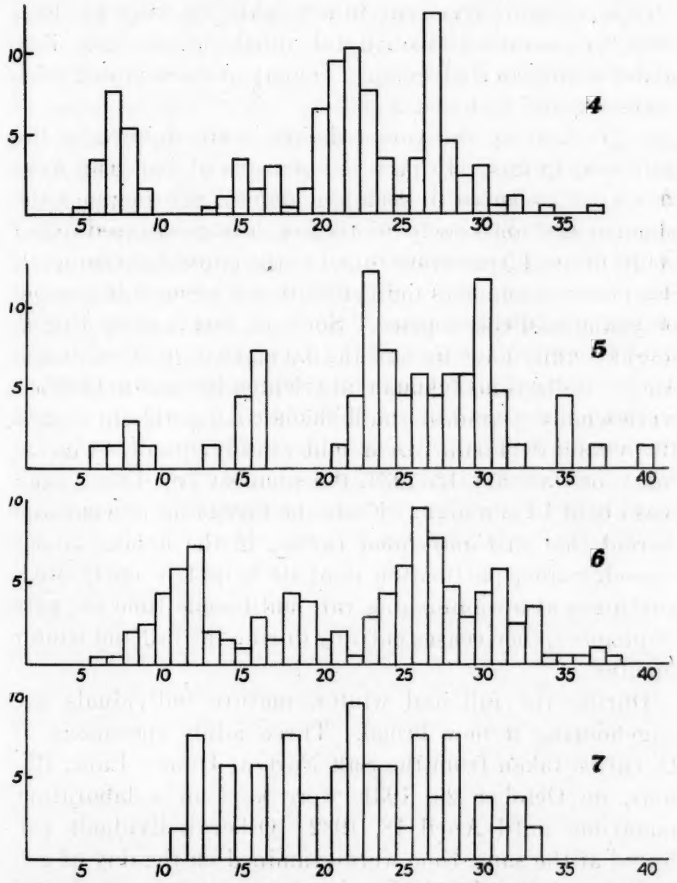

FIG. 4. A population sample taken on July 3, 1934, showing an exceptionally sharp distinction between age groups. Growth has apparently been somewhat retarded in the two-year group with mode at 27, for normally this group is above 30. FIG. 5. A late summer population sample, near the close of the parturition period, August 13, 1932. FIG. 6. A characteristic late summer or early fall population sample, September 5, 1932. FIG. 7. A fall sample, after the close of the parturition period, October 21, 1933.

period of approximately six months in the laboratory, no young were liberated, though the snails were active and apparently normal in every respect. This experiment

checks with observations in the field, for no young of a size comparable to the largest uterine young have been taken in any of the stream collections between September 29 and May 15.

Call (1886: 165) expressed the general belief that for members of this genus the young are extruded from April to August and further stated that "there is reason to believe that there are often two broods in the year." While the present study has corroborated the former of these two statements for *C. rufum,* neither field collecting nor studies of the uterine young support the supposition that two broods are produced annually. The prolonged period over which young are being liberated tends to eliminate sharp lines of demarcation between broods. Incidental field collecting might readily yield the impression that the young born in September represent a distinct brood, though in fact they represent but the extreme limit of a single, prolonged birth period.

TABLE II

SEASONAL VARIATION IN PERCENTAGE OF GRAVID INDIVIDUALS IN THE MINIMUM SIZE GROUP OF BEARING INDIVIDUALS OF *Campeloma rufum*

Date	Smallest size group bearing either eggs or young	Percentage of this group bearing eggs or young
Apr. 15, '34	20–24 mm	30
May 15, '32	20–24 mm	30
May 19, '34	15–19 mm	57.1
June 4, '32	20–24 mm	70
June 25, '32	20–24 mm	100
Aug. 13, '32	15–19 mm	67
Sept. 1, '33	(15–19 mm)	(2)
	20–24 mm	53.5
Oct. 21, '33	20–24 mm	53.7
Jan. 1, '34	20–24 mm	61.9

It seems apparent, from observations at hand, that size is not the sole criterion for marking the advent of maturity, since there is a definite seasonal variation in size of the smallest mature individuals (Table II). On August 13, 1932, 67 per cent. of all the 15–19 mm shells bore either eggs or young, but on September 1 of the following year less than 2 per cent. of the 15–19 mm shells were gravid. It thus seems that while 20 mm is the usual lower limit for gravid individuals, this class may vary somewhat in

correlation with seasonal and other environmental conditions.

The percentage of barren individuals varies widely through the year. In routine practice all empty uteri were recorded as barren though, obviously, many of the snails were too young to produce eggs or young. In consequence, a correlation factor (Table III) was introduced to secure the net number of barren individuals for each sample. Previous calculations (Table II) had

TABLE III

INCIDENCE OF BARRENNESS IN *Campeloma rufum*, COMPUTED ON A NET BASIS BY DEDUCTING THE IMMATURE YOUNG FROM THE TOTAL NUMBER OF INDIVIDUALS HAVING EMPTY UTERI IN THE POPULATION SAMPLE

Date of collection	Number with empty uteri			Percentage of barrenness in total population sample
	Total	Immature	Net number barren	
May 15, '32	25	7	18	54
June 4, '32	13	3	10	40
June 25, '32	22	0	22	44
Aug. 13, '32	19	0	19	36.8
Sept. 5, '32	29	11	18	24
Oct. 21, '33	24	19	5	12.5
Mar. 3, '34	8	8	0	0
Apr. 15, '34	99	93	6	4.11
May 19, '34	10	5	5	8.9

demonstrated the minimum size group for gravid individuals for each sample. Empty uteri in shells below this size were eliminated to secure the net figures for barrenness. It was found that the highest incidence of barrenness occurred in May, 1932, when 54 per cent. of shells over 20 mm in height had empty uteri. During the summer the incidence of barrenness decreases rapidly until by mid-winter and early spring practically all shells above 20 mm in height carry either eggs or a brood of young.

Leaving out of consideration the abnormal young, which according to Mattox (1935: 144) are not uncommon in uteri of this species, the normal young carried by a gravid parent range in size from about 1.5 mm to 4.5 mm in shell height. Several thousands of uterine young have been measured, including samples for every season of the year (Table IV). Most of these samples contained more than

fifty young, while several samples have contained more than 300 embryonic shells. Throughout most of the year the uterine young conform to a fairly normal distribution curve when plotted on shell height.

TABLE IV

A SEASONAL STUDY OF RANGE IN SIZE OF THE UTERINE YOUNG OF *Campeloma rufum*. FOR EACH COLLECTION THE MODE IS INDICATED BY ITALICS AND THE MEDIAN BY AN ASTERISK

Height class of embryonic shells in mm.	Apr. 23, 1932	May 15, 1932	May 19, 1934	Aug. 13, 1932	Sept. 1, 1933	Sept. 5, 1932	Sept. 29, 1931	Sept. 30, 1930	Oct. 21, 1933	Oct. 25, 1931	Jan. 13, 1934	Mar. 3, 1934	Apr. 15, 1934
1.05–1.54	0	0	0	0	0	0	0	0	0	1	0	0	4
1.55–2.04	6	0	3	2	4	2	2	9	4	4	2	1	2
2.05–2.54	24	4	5	*17**	20	16	49	43	14	33	1	5	18
2.55–3.04	87	19	1	8	54	29	143	85	22	76	9	6	32
3.05–3.54	*159**	12*	15	1	*98**	*46**	*184**	*149**	40*	*127**	*26**	39	*105**
3.55–4.04	65	*24*	*31**	0	64	18	126	41	*59*	109	16	*71**	104
4.05–4.54	2	0	5	1	10	1	6	2	8	17	3	8	21
4.55–5.04	0	0	0	0	0	0	0	1	0	1	0	0	0
	343	**59**	**60**	**29**	**250**	**112**	**510**	**330**	**147**	**368**	**57**	**130**	**288**

The only direct evidence of periodicity in repopulation of the uterus is found in the fact that a distinct instability of the mode for height of uterine shells starts in April (Table IV) and does not become stabilized until September. Thereafter and for most of the year the mode for uterine shell height lies in the 3.05 to 3.54 mm class. As previously stated, relatively large numbers of young are born in May. Correlated with this we find that the uterine young of uteri examined in May approach the maximum size with a mode a half millimeter larger than that characteristic for most of the year. Immediately following the parturition period the mode recedes to 3.05 to 3.54 mm class, where it remains throughout the winter with a fair degree of stability.

When this study of uterine young was first undertaken, it was assumed that the uterine population would increase progressively in shell size up to the advent of the parturition period, and this would be followed by a period characterized by empty uteri. This assumption was based upon the supposition that a restricted parturition period would be followed by a repopulation of the uterus by embryos of approximately uniform age. But field obser-

vations on the young in the stream and measurements of the uterine shells failed to support this assumption. Except for the influence of the spring liberation of young, the uterine population remains remarkably uniform in character (Table IV) showing little progressive change in the embryonic population as a whole throughout most of the year.

TABLE V

AN ANALYSIS OF THE RELATIONSHIP BETWEEN SIZE OF PARENT SHELL AND PRODUCTIVITY IN *Campeloma rufum*. DATA FROM SELECTED SAMPLES, NOT FROM ENTIRE POPULATION SAMPLES

Size of parent shell in mm.	Average number of eggs in uteri bearing eggs	Average number of young in uteri bearing shelled young
10–14	0	0
15–19	2	1
20–24	7.2	4.6
25–29	4.4	15.7
30–34	8.2	13.8
35–39	8.7	33.4

In Table V, a series of eight collections, representing different seasons of the year, were thrown together to determine any relationship which might exist between size of the parent and number of eggs and young borne. It is evident that in this species there is an increase in capacity for bearing young, directly correlated with increase in size of the parent snail. There is a pronounced, sudden increase in average number of young between the 20–24 mm group and the 25–29 mm class. Between these limits fall the limitations of size usually attained by one-year-old individuals. It is thus evident that *C. rufum* begins to bear eggs and young in the uterus before attaining an age of one year but enters a period of greater productivity upon attaining the age of one year and reaches its maximum in the second or third year.

The maximum number of uterine young (Table VI) encountered in this study was 90 in the uterus of an individual having a shell 36.4 mm high, collected on September 29, 1931. As stated elsewhere in this report, the young engendered in late summer and early fall are carried in the uterus through the winter. Correlated with this fact is the observation here made that uterine young are much

TABLE VI

SEASONAL VARIATION IN THE SIZE GROUP OF ADULTS OF *Campeloma rufum* HAVING THE INDIVIDUAL BEARING THE LARGEST NUMBER OF UTERINE YOUNG

Date	Size class of parent shell in mm.	Number of young in the most prolific individual
Mar. 3, '34	30–34	29
Apr. 15, '34	25–29	27
May 5, '32	30–34	23
May 19, '34	25–29	14
June 25, '32	30–34	4
Aug. 13, '32	35–39	7
Sept. 5, '32	30–34	16
Sept. 29, '31	35–39	90
Oct. 21, '33	25–29	27

more numerous in fall and winter than in late spring and summer. At the close of the parturition period the number of uterine young drops off abruptly and the uteri become ready to start a new cycle of developing eggs. Thus in a June sample (Table VI) four embryonic shells were the most found in any one uterus and many of the uteri were wholly spent. During the early summer, when uterine young are at their lowest ebb, the uterus becomes filled with eggs. From these eggs are produced the young that will be liberated the next spring (May). Detailed information on the rate at which eggs enter the uterus is wholly lacking. Through summer and fall the relative numbers of eggs and young vary widely in different individuals. Either eggs are produced over a relatively long period of time or else individual eggs have pronounced differences in the rate of embryonic development. At least some evidence in favor of the latter is found in the fact that polyvitelline eggs were observed in which two young shells within the same membrane manifested extreme differences in size (see Mattox, 1935, Fig. 2). Since the eggs entering the uterus in the summer are not discharged as fully developed snails until the following May, the period of embryonic development must cover approximately nine months.

Contrasted with the record of 90 young in a single uterus, the number of eggs present at one time is distinctly low. In the uterus of one 36.9 mm snail taken on August 13, 1932, there were 21 eggs present. No other

specimen examined in the course of this investigation contained more than 20 eggs, while at most seasons of the year the maximum number of eggs in bearing individuals was distinctly below 20 per uterus.

Summary

(1) *Campeloma rufum* in the Salt Fork River at Homer Park, Illinois, has been subjected to field and laboratory analysis of population samples taken at irregular intervals over a period of four years.

(2) Collections of active snails have been taken at all seasons of the year, including winter.

(3) Contrary to all recorded observations, separate sexes do not occur in *C. rufum* in the habitat under consideration.

(4) More than 1,500 specimens representing every season of the year have been examined critically, but neither males nor protandrous hermaphrodites with any sort of copulatory organs have been found. All available evidence points to parthenogenesis for this species.

(5) This is the first recorded instance of lack of males in the genus *Campeloma*.

(6) Shells of uterine young range in height from about 1.5 to 4.5 mm.

(7) Parturition is most active in May but extends through summer to about September 1.

(8) Field collections and adult snails kept in laboratory aquaria over winter give evidence that young are carried in the uterus through the entire winter without any being liberated.

(9) The new generation born in May has a mode of shell height of 4 mm. By September, these have reached about 12 mm. By the following May, at the age of one year, these have reached about 22 mm.

(10) The smallest bearing individuals are about 20 mm in shell height.

(11) In a number of collections a second mode of distribution occurs at about 30 mm. These individuals seem to be two years of age.

(12) Forty mm is the maximum for *Campeloma rufum* in the habitat under consideration. These largest individuals are interpreted as probably three years of age.

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SHORTER ARTICLES AND DISCUSSION

A CASE OF HIGH MUTATION FREQUENCY WITHOUT ENVIRONMENTAL CHANGE

STUDIES of mutation frequency in *Drosophila melanogaster* have shown that a certain rate of mutation may be expected if large numbers of individuals are tested under constant environmental conditions. This mutation rate varies with different external environments and is different from gene to gene as well as for the same gene in different strains (Timofeef-Ressovsky, 1932). While we know little of mutation rates in nature, there are ample data to show that under constant optimal laboratory conditions the same strain gives a certain basic rate of total mutation for all genes. For instance, it has been found in our laboratory that at 24° C under constant conditions of light and moisture, X lethals appear in Fla stock at about the rate of 1 lethal per 100 chromosomes tested, while for all easily recognized visible mutations the numbers are from 3 to 5 per 100,000 flies examined (Plough and Ives, 1935). Approximately similar figures may be arrived at from the data of other investigators. They constitute what are called the "spontaneous" mutations and may be supposed to account for genetic variance in nature.

Spencer (1935) has recently described the results of eight years' search for mutations in *D. funebris* and *D. hydei,* during which more than 750,000 flies were examined. His total frequency ($\pm$ 5 per 100,000) is not far from that given above for *D. melanogaster,* but the significant point is that it is not uniform in time. These mutations occurred in two mutating periods of about 30 months each, separated by a three-year period during which not a single visible mutation was found. From the coincidence of these mutating periods in the two species he is inclined to postulate unknown extrinsic factors in the environment as the causal agents in the production of these mutations. It seems of interest, therefore, to describe a case of high mutation frequency in a strain of *Drosophila melanogaster,* where parallel experiments gave no such increased frequency, and where apparently the increase in mutation rate can not be supposed to be due to extrinsic factors.

On December 7, 1935, the junior author was given a bottle containing 3 virgin wild type females (Selected line B Fla No. 7) mated to 3 black-purple-curved males, and he was asked to make a back cross of the F_1 females to homozygous recessive males from stock to determine the percentages of crossing over. The

back cross was made on December 23, and on January 8 a first count was made of the segregating offspring. Among these were found 7 individuals out of about 300 showing the mutation blistered wing. Three blistered females were crossed to several normal males of the same generation, and their offspring, inbred for six successive generations, are the material of the rather unique history here reported. In each of these inbred generations about 30 cultures were run and in each a number of variants appeared and were tested as mutations. In the last two generations the fertility markedly decreased and when the stock was outcrossed to the original wild stock to recover fertility no further mutations appeared.

Table 1 summarizes the results obtained in this series of matings derived from inbred blistered flies.

TABLE 1

First appeared in Generation No.	Non-inherited	Mutation and location	Sterile (possibly genetic)
1		Blistered II	
2	Notch wing Rough eye	Star-like eye II	Short bristles Stubby bristles
3	Bristle mosaic Cut wing Extra bristles	Lace wing II (extreme plexus) Abnormal abdomen	Minute
4	Unsymmetrical wings	Scute— new allele I	Club wings
5		Extra d c bristles	Rough eye Short bristles
6			
7	Extreme notch wing		Swollen or bulge eye
Totals	5	6	6

Most of the 17 variants listed occurred several times, and in all 35 were tested. Thus in a total of 175 cultures or about 30,000 flies derived by inbreeding from the original blistered individuals 6 mutations were isolated, and as many more sterile individuals—probably genetic in origin—were found. Thus this series of matings gave a mutation frequency from 3 to 6 times greater than that usually found for Drosophila cultures under constant environmental conditions.

The uniqueness of this result is proved by comparison with other Drosophila experiments being run in the laboratory at the same time. The bottles were not run in the constant temperature room, but were kept on the shelves of the genetics laboratory, where the temperature averaged 22° C, with a range of as much as 5° during the day. During the same period 14 other members

of the genetics class were running the same initial cross in the same place, and while several tested possible mutations, not a single one was found. More significant as controls of this particular series of matings were the hundreds of tests for lethal and other mutations being run by Dr. George Child and the senior author in the constant temperature room across the hall. As will be reported in another place, these tests gave mutation frequencies closely following the temperature, and with no indication of increased frequency during the period covered by the experiments here reported.

Had unknown extrinsic factors of the sort suggested by Spencer been operating, it would seem as though some general increase in mutation frequency should have shown itself in the hundred thousand or more flies examined during the three months' period beginning with January 8. The complete lack of any such coincident increase in the mutation frequency in the simultaneous experiments suggests that the "mutating period" in this line was due to intrinsic rather than extrinsic causes.

The simultaneous demonstration by Demerec (1936) of the presence of a mutability stimulating factor in Florida stock gives further weight to our conclusion that this "mutating period" was initiated by a genetic change within a single individual.

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ADDITIONAL EVIDENCE OF REPEATED CHROMOSOME DIVISION WITHOUT MITOTIC ACTIVITY

SINCE the time of Weismann it has been known that the growth of many insect tissues during the larval period is due to increase in size, not in number, of cells. Jakobj (1925) reported that this increase in size comes about by repeated doubling in volume. He was of the opinion that volume increase was initiated by division of all the "protomeres" in the nucleus and their subsequent

growth to the original size. The same general idea of cell growth by "inner division" was advanced by several other investigators, notably by G. Hertwig (1935).

Hertwig suggested that after a cell division the nuclei and chromosomes of the daughter cells have the faculty of growth by doubling of the "genome" and of the nuclear volume. Only at the end of such a doubling process is the cell again ready for mitosis. If the mitosis does not occur, as in the case of the larval tissues under consideration, further growth may take place by further doubling of the genome and of the nuclear and cell volumes. This process gives rise, according to Hertwig, to large nuclei with "multivalent" chromosomes. Applying this idea to the cells of insect salivary glands and by measuring the volume of these cells he arrived at the conclusion that the huge salivary gland chromosomes have a "valence" of 256 or 512, *i.e.*, these giant chromosomes are compound structures in which the original gene complex is present 256 or 512 times.

The evidence for "internal division" of chromosomes has thus far been derived largely from measurements of resting nuclei and comparison of their volumes. Hertwig uses the theory of "internal division" as evidence for the multivalence (Vielwertigkeit) of salivary gland chromosomes. Since it has not been demonstrated that these large chromosomes do actually consist of several hundred "genomes" this possibility does not form conclusive proof of growth by inner division.

The case reported below is one which seems clearly to involve an actual increase in the number of chromosomes by repeated divisions during the growth of nuclei that appear to remain in a typical "resting stage" condition. This occurs in the epithelial cells of the larval ileum of the mosquito *Culex pipiens*.

The ileum of *Culex* is composed of two tissues, an outer circular muscle layer and an inner single-celled epithelial layer. The epithelium of the ileum grows throughout the larval period by increase in cell size. The number of cells remains constant during the larval stage and the first ten hours of pupal life. Cell counts for this tissue varied between 119 and 139 cells in different individuals.

In young first instar larvae the diameter of the nearly spherical nuclei of these cells ranges from 3 to 4 micra, the same as that of the regenerative nuclei of the mid-gut. At the end of their growth period these same nuclei are in the shape of flattened ellipsoids with their three axes roughly in the proportion of 4:2:1. Though all the nuclei are large at this stage, they are not all of

uniform size, and measurements of their long axes range from 10 to 17 micra. These measurements are not incompatible with three or four doublings of the original volume. Throughout the larval stage these nuclei maintain the appearance of typical resting nuclei. The chromatin is present in the form of delicate, faintly granulated threads, giving the general appearance of a reticulum. No heavy chromosomes of the type found in salivary glands are present.

The metamorphosis of the ilial epithelium begins at the tenth hour of pupal life and is peculiar in that it does not take place through the agency of an imaginal ring or of scattered regenerative cells, but by the division and multiplication of the larval epithelial cells. At metaphase of the first cell divisions initiating metamorphosis the multiple chromosome complexes first reported by Holt (1917) appear. The diploid chromosome number for *Culex* is six. The number most commonly found at metaphase in the first division of metamorphosis is 48, more rarely and only in the largest of the cells 96 chromosome metaphases are found.

These multiple chromosome complexes have apparently arisen during the larval growth stage by three or four successive divisions of all the chromosomes while in the diffuse or "resting stage" condition. Since the epithelial cells of the newly hatched larva do not divide it is impossible to demonstrate directly that they have normal diploid (6 chromosome) nuclei. The nuclei of these young cells, however, have the same size and appearance as the regenerative nuclei of the mid-gut which do undergo mitosis and are clearly seen to have 6 chromosomes. Furthermore, the space occupied by 96 or even 48 chromosomes is such that they could not be packed into a nucleus of 3 to 4 micra in diameter, even though the size of an individual chromosome of a 96 complex does not differ materially from that of a member of a 6 chromosome complex. Again, if the tissue was polyploid at the time of hatching we should expect the same degree of polyploidy in all the cells, since they are quite uniform in size at this period. On the other hand, if polyploidy arises as a result of growth and repeated chromosome division the different numbers of chromosomes in the multiple complexes are comprehensible on the assumption of slight differences in growth rate or in the nutrition of different cells. This view is supported by the fact that the number of chromosomes in the complex appears to be directly correlated with the size of the nucleus.

As the metamorphosis of the ileum progresses, repeated cell division results in an increase in the number of cells and a

decrease in their size. Multiple complexes of 24 and of 12 chromosomes are commonly found at this stage and again the chromosome number is correlated with nuclear size. It appears that a process of somatic reduction of chromosome number takes place during the rapid metamorphosis of this tissue. Whether the relatively small nuclei of the rebuilt imaginal ileum are tetraploid, as reported for other insects by Frolowa (1929), or diploid can not be said at present. The whole question of the fate of the multiple complexes is now being investigated.

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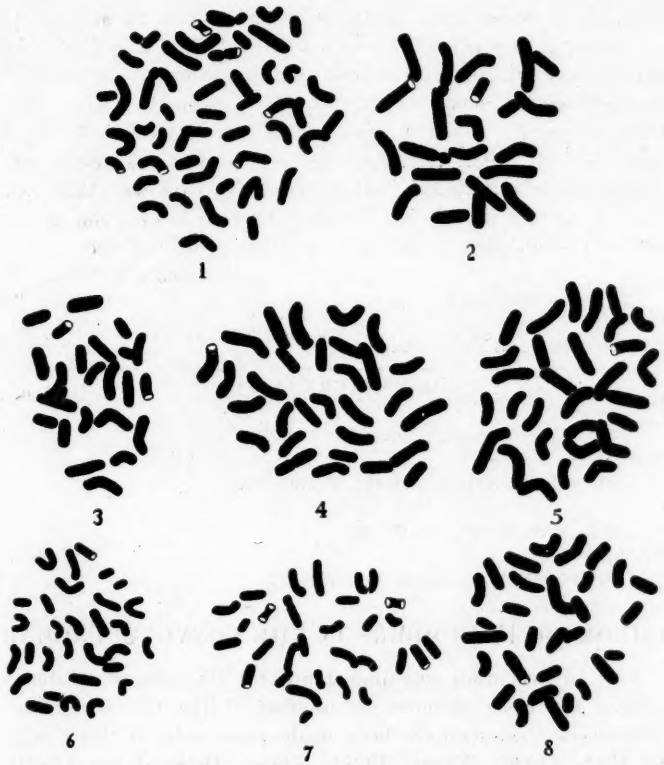
CHROMOSOME NUMBERS IN THE CONVOLVULACEAE

THIS investigation was undertaken for the purpose of determining the basic chromosome number of the Convolvulaceae. Chromosome counts have been made previously in this family by Heitz (1926), Yasui (1928), Nagao (1928), Kano (1929), Nakajima (1931) and Sugiura (1931). Definite diploid numbers of 20, 22, 28, 30, 44 and 58 have been reported.

The sources of the seeds used in this work are shown in Table I. A tuber of *Ipomoea pandurata* furnished the material for that species. Seeds obtained from commercial seed houses were grown and the species determined. The remaining forms were identified from the plant producing the seeds. Seeds were germinated on moist filter paper. Root tips were fixed in Nawaschin's solution, embedded in paraffin and sectioned at 10 and 12 microns. Some preparations were stained according to Newton's iodine-gentian violet method. Smith's (1934) modification of this method gave better results. Drawings were made, with the aid of a camera lucida, at a magnification of 5500×.

Chromosome numbers of seven species of the Convolvulaceae were determined for the first time (Figs. 1–7). Six species which had been counted prior to this time were checked. One



FIGS. 1–8. Metaphase plates from root tips: fig. 1, *Convolvulus arvensis* with 50 chromosomes; fig. 2, *C. spithamaeus*, 22; fig. 3, *C. sepium*, 24; fig. 4, *Ipomoea saggitata*, 30; fig. 5, *I. pandurata*, 30; fig. 6, *I. carolina*, 30; fig. 7, *I. lacunosa*, 30; fig. 8, *Ipomoea* (hybrid ?), 30. ×4000.

unidentified form which morphologically appears to be a natural hybrid between *Ipomoea purpurea* and *I. hedereacea* was also studied (Fig. 8). Only somatic tissue of this form has been studied and as yet no genetic analysis has been attempted. The results are given in Table I. Those species previously investigated are followed by the name of the investigator.

No basic number has been assigned to the Convolvulaceae. Heitz (1926) suggested that *Convolvulus siculus* (2n = 44) may be a tetraploid form of *C. elongatus* (2n = 22) or of *C. undulatus* (2n = 22). *C. spithamaeus* (2n = 22) would fit into such a series, but other species of Convolvulus, with diploid numbers of 20 (Heitz, 1926), 24, 30 (Nakajima, 1931) and 50, would not. In

TABLE I

Species	2n Chromosome number		Source of material
Ipomoea purpurea Roth. (*Pharbitis hispida*, of Kano)	30	Kano '29	Charlottesville, Va.
I. hederacea Jacq. (*Pharbitis hederacea*, of Kano)	30	Kano '29	Stumpp and Walter
I. lacunosa Linn.	30		Keysville, Va.
I. carolina Pursh.	30		Charlottesville, Va.
I. saggitata Cav.	30		Wrightsville Beach, N. C.
I. pandurata Meyer	30		Keysville, Va.
I. setosa Ker.	30	Nakajima '31	Stumpp and Walter
I. rubro-caerulea Hook.	30	Nakajima '31	Stumpp and Walter
Calonyction aculeatum House (*Convolvulus aculeatus* Hill)	30	Nakajima '31	Stumpp and Walter
Convolvulus arvensis Linn.	50		Charlottesville, Va.
C. sepium Linn.	24		Eatontown, N. J.
C. spithamaeus Linn.	22		Charlottesville, Va.
Quamoclit coccinea Moench.	28	Nakajima '31	Keysville, Va.
Q. coccinea (cultivated)	28	Nakajima '31	Vaughan's Seed Co.
Ipomoea (hybrid?)	30		Charlottesville, Va.

the genus Ipomoea, including Quamoclit species, the diploid numbers 28 and 30 suggest aneuploidy rather than a regular polyploid series. The basic chromosome number, or numbers, can not be determined without further investigation.

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Errata: The title of the article by Professor A. H. Sturtevant and Professor Th. Dobzhansky in the issue of THE AMERICAN NATURALIST for November–December, p. 574, should read: "Observations on the species related to *Drosophila affinis*, with descriptions of seven new forms." On page 576, fourth line; "♀ Abdomen indistinctly banded," should read "♀ Abdomen distinctly banded."